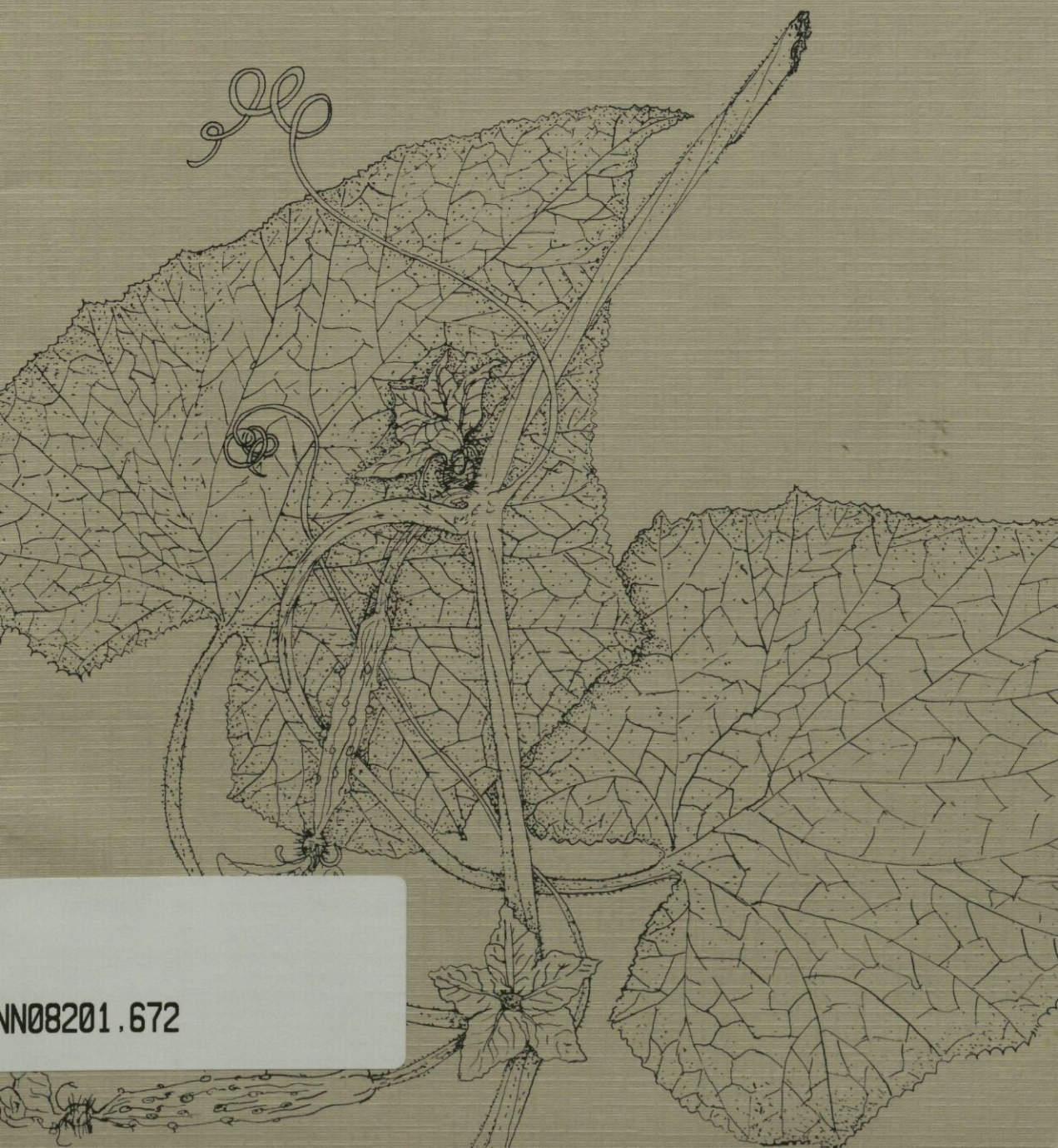
An analysis of the diurnal course of growth, carbon dioxide exchange and carbohydrate reserve content of cucumber

H. Challa



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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. ir. J.P.H. van der Want, hoogleraar in de virologie,
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Abstract

Challa, H. (1976) An analysis of the diurnal course of growth, carbon dioxide exchange and carbohydrate reserve content of cucumber. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 861, ISBN 90 220 0623 9, (viii) + 88 p., 28 tabs, 32 figs, 72 refs, Eng. and Dutch summaries.
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A detailed study was made of the diurnal course of carbon dioxide exchange, transpiration and carbohydrate reserve levels in different organs of young cucumberplants, cultivated in climate rooms under 'spring' or 'winter' conditions. Under spring conditions the stomata closed after about 8 hours light, causing a decrease in the rate of CO_2 uptake. This closure could not be ascribed to water shortage. Under winter conditions CO_2 production rapidly decreased after about 12 hours darkness, as a result of carbohydrate depletion. Additional respiration substrates were provided by protein breakdown. Reducing the air temperature during that period from 25 °C to 12 °C caused an increase in rate of plant growth, probably by reducing the amount of protein breakdown. The moment at which starch reserves were depleted was, under the conditions studied, independent of the amounts formed and seems to be 'preprogrammed'. Majority of the carbohydrate reserves formed during the day were used as respiration substrates. A comparison of the measured amounts of CO_2 production with theoretically derived values, showed a discrepancy which may be explained by underestimation of the amount of protein turnover.

Furthermore a new method is described for calibration of differential water vapour analysers and also for mixing pure CO_2 with CO_2-free air to obtain air mixtures with various constant CO_2-concentrations.

Keywords: energy saving, glasshouse, climate, cucumber, stomata, transpiration, photosynthesis, respiration, starch, sugars, circadian rhythms, protein turn-over, calibration methods, growth.

This thesis will also be published as Agricultural Research Reports 861 and as Publication No 020 of the Centre for Agrobiological Research (CABO), Wageningen.

Stellingen

1. Bij de klimaatregeling in de kasteelt houdt men onvoldoende rekening met het dagelijkse verloop van fysiologische processen in de plant.

Dit proefschrift.

2. Een kasklimaatregeling waarbij van moment tot moment de koolzuuropname van het gewas wordt gemaximaliseerd, leidt tot lage opbrengsten.

T. Takakura (1973). Acta hort. 46: 147-151.

3. Het begrip onderhoudsademhaling wordt door verschillende auteurs inconsequent gebruikt.

K.J. McCree (1974). Crop Sci. 14: 509-514.
F.W.T. Penning de Vries (1975). Ann. Bot. 39: 77-92.
G.J.A. Ryle, J.M. Cobby & C.E. Powell (1976). Ann. Bot. 40: 571-586.

4. Volgens Ludwig et al. is bij verandering van de CO_2 concentratie het verband tussen CO_2 opname tijdens de dag en CO_2 productie tijdens de nacht bij tomatenbladeren niet het zelfde als bij verandering van de lichtintensiteit. Een dergelijk verschil is te verwachten wanneer de gehele plant wordt blootgesteld aan veranderingen in lichtintensiteit en slechts een enkel blad aan veranderingen in CO_2 concentratie.

L.J. Ludwig, D.A. Charles-Edwards & A.C. Withers (1975). In: R. Marcelle (ed.), Environmental and biological control of photosynthesis, Dr. W.J. Junk N.V. Publishers, The Hague, p. 29-36.

5. Ter besparing van energie kunnen warmtebehoeftige gewassen gedurende de wintermaanden beter verbouwd worden in goed geisoleerde ruimtes met kunstlicht, dan in kassen. De daarmee gepaard gaande investeringen zijn echter groot, zoals vaak het geval is bij energiebesparing.

P. Gaastra (1974). Bedrijfsontwikkeling 5: 889-897.

6. Het gebruik van 'total energy' systemen in de glastuinbouw moet worden gestimuleerd. De medewerking van de regionale electriciteitsmaatschappijen is hierbij vereist.

7. Bij het bestuderen van de assimilatendistributie in de plant met $^{14}CO_2$ moet rekening worden gehouden met de inbouw in eiwitten en organische zuren.

A.A. Khan & G.R. Sagar (1969). Ann Bot. 33: 753-780.

8. Het begrip kwaliteit in de Nederlandse tuinbouw is onvoldoende afgestemd op de behoeften van de consument.

9. De volkstuin heeft ondermeer door zijn kleinschaligheid een grote landschappelijke waarde en zou daarom meer dan thans zijn plaats binnen de stedelijke bebouwing moeten vinden.

10. Het vele geld dat bij 'doe-het-zelven' in en om de woning wordt gespendeerd, rechtvaardigt een actief voorlichtingsbeleid van de overheid op dit terrein.

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Opgedragen aan hen, die door hard werken de oogst van de zon aan de schoot van moeder aarde ontworstelen.

Levensloop van de auteur

De auteur werd op 13 juli 1944 geboren in Voorburg. In 1962 beëindigde hij de HBS-b opleiding aan het Montessori Lyceum in Den Haag en begon toen zijn studie aan de Universitá degli Studi van Milaan, aanvankelijk aan de biologische faculteit, later aan de faculteit van de landbouw. In 1964 zette hij deze studie voort aan de Landbouwhogeschool in Wageningen en koos daarbij de richting bosbouw (houtteeltkundig). Met het vakkenpakket houtteelt, plantenfysiologie, theoretische teeltkunde en wiskunde rondde hij de studie af in 1971 en kwam toen als promotieassistent in dienst van het Centrum voor Plantenfysiologisch Onderzoek (CPO). Op voorstel van de directeur van dat instituut, dr. ir. P. Gaastra, begon hij een onderzoek ten behoeve van de optimalisering van de klimaatregeling in de kasteelt. De begeleiding van dit onderzoek lag in handen van prof. dr. ir. C.T. de Wit en later ook van prof. dr. R. Brouwer. In 1973 werd de auteur opgenomen in de vaste personeelsformatie van het CPO. Als gevolg van het samengaan van het CPO met het IBS in 1975 is hij thans verbonden aan de afdeling fysiologie van het uit deze fusie voortgekomen Centrum voor Agrobiologisch Onderzoek (CABO).

Voorwoord

Aan het tot stand komen van dit proefschrift hebben velen een bijdrage geleverd. De plezierige samenwerking waarin dat plaats vond is voor mij een waardevolle ervaring geweest. Zij die daarbij het meest betrokken zijn geweest zou ik met name willen noemen.

Dr. ir. P. Gaastra heeft met zijn suggestie voor het onderwerp van dit proefschrift gezorgd voor een boeiende en verfrissende probleemstelling. Voorts heeft hij een welhaast ideaal werkklimaat weten te scheppen door de uitstekende faciliteiten die mij geboden werden, en door de vele contacten die hij voor mij wist te leggen.

Prof. dr. ir. C.T. de Wit heeft met zijn actieve en kritische begeleiding een waardevol stuk wetenschappelijke vorming voor zijn rekening genomen. Dikwijls waren het de discussies met hem, die een lichtpunt deden ontstaan op momenten waar het onderzoek stagneerde.

Prof. dr. R. Brouwer heeft vanuit zijn visie mijn onderzoek in verschillende fasen doorgelicht en zijn opmerkingen zijn daarbij, mede door zijn grote kennis van de literatuur, van grote waarde geweest.

Bijzonder grote erkentelijkheid zou ik willen uitspreken voor de enorme inzet waarmee, vooral in de eindfase, de totstandkoming van het manuscript door bovengenoemden is begeleid. Ondanks hun zeer druk bezette leven wisten zij steeds weer tijd, hetgeen meestal inhield privé-tijd, vrij te maken om gereedgekomen onderdelen van het manuscript direct door te nemen en te bespreken. De plezierige sfeer waarin deze besprekingen plaatsvonden maken deze tot een prettige herinnering.

Een grote bijdrage aan dit proefschrift leverde Janneke Elema en de heer P. Brouwer door de zorgvuldige uitvoering van de experimenten en door hun inspirerende belangstelling voor dit onderzoek. Voorts zou ik willen bedanken:
- de heer C.R. Vonk en zijn medewerkers voor de zetmeel- en suikergehaltebepalingen,
- dr. W. Dijkshoorn en drs. N. Vertregt en hun medewerkers voor de chemische analyses,
- de medewerkers van de werkplaats van het voormalige CPO voor het vervaar-

digen van talloze onderdelen voor de verschillende meetopstellingen en voor de vele assistentie bij het verhelpen van storingen,
- de medewerkers van de vakgroep Theoretische Teeltkunde van de Landbouwhogeschool voor hun waardevolle adviezen. In het bijzonder dr. F.T.W. Penning de Vries ben ik zeer erkentelijk voor zijn suggesties en raadgevingen. Zijn pionierswerk vormde een belangrijke basis voor dit proefschrift,
- dr. K. Schurer van de TFDL voor zijn metingen van lichtabsorptiekarakteristieken van bladeren,
- de medewerkers van het Proefstation voor de groente- en fruitteelt onder glas te Naaldwijk voor de waardevolle suggesties en discussies,
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- de typekamer van mevrouw A.E.Ch. van Vliet-Touburg van het BGD voor de zorgvuldige uitvoering van het typewerk,
- Erica Brouns-Murray en Bram van der Zalm van het Pudoc voor hun bijdrage bij de vormgeving van dit proefschrift,
- Jaap Uittien voor het ontwerp van de omslag,
- mijn collega's voor hun interesse in de voortgang van het werk en de bereidheid tot opmerkingen en discussies.

Verder dank ik dr. ir. G.A. Pieters van de afdeling Plantenfysiologisch Onderzoek van de Landbouwhogeschool, voor de uitstekende vorming, die hij mij in de ingenieursfase van mijn studie heeft gegeven en waarop ik talloze malen heb kunnen terugvallen.

Last but not least in deze rij van namen zou ik graag mijn vrouw noemen. Hermien, jou dank ik voor het overnemen van al die taken, waaraan ik zelf niet toe kwam en voor het actieve meebeleven van hoogte- en dieptepunten tijdens de afgelopen periode.

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1 General introduction

Cultivation of subtropical crops such as cucumber, tomato, pepper and eggplant during the winter in countries like the Netherlands, Great Britain and Germany, requires much energy. Energy requirements are high because the difference between the optimum temperature of these crops and the average outside temperature is considerable. Moreover daylength and average irradiance available for growth are small resulting in a low growth rate.

Since the oil crisis of 1974 energy prices have risen, and will probably go on increasing. Increased costs and the general opinion that energy resources have to be spent carefully, lay a heavy burden on the shoulders of the glasshouse grower, who is expected to produce the same quantity and quality but use less energy.

Energy consumption in glasshouse crop cultivation may be reduced by technical measures (Germing, 1974; Gaastra, 1974) like increasing the efficiency of the heating system, or decreasing heat exchange between glasshouse and environment. But this aspect will not be dealt with in this study.

Much energy could be saved if air temperature in the greenhouse were lowered even by a few degrees. Germing (1974), for example, calculated that lowering the average air temperature in early tomato production by 1 ^{o}C could result in a decrease of energy consumption of 9% from January till July. After many years of research on the optimum temperature for different crops, it may be expected, however, that temperature decrease, applied in the usual cultivation systems, will tend to decrease productivity.

Thus we come to the conclusion that decreasing temperature in glasshouse production, without affecting productivity, means that climate will have to be controlled in a different way from existing methods. Modern climate controls keep the night temperature at one fixed value, the day temperature at another, a few degrees higher, and allow a certain increase in air temperature with increasing irradiation (Germing, 1969). Most refinements have been found in the optimization of combinations of pipe temperature and amount of ventilation (window opening), which however mainly control water vapour pressure deficit and thus crop transpiration.

At this point one may ask whether temperature requirements of different crops are indeed constant under various environmental conditions, or at different moments of their day/night cycle. It should be realised that not only external factors like irradiance and concentration of carbon dioxide may change dramatically, but also internal levels of reserves, hormones and enzymes (Upmeyer & Koller, 1973; Zucker, 1972; Hewett & Wareing, 1973).

With detailed information about temperature requirements of crops in course of time, energy could be saved and yields increased. Highly sophisticated control systems have been developed in greenhouse technology, with which it is possible to adapt greenhouse climatization almost instantaneously to the outside conditions. Moreover the recent introduction of process computers for climate control makes it possible to store data collected over a certain period and to use them in further programming. With such a system the control program can be adapted to situations which occurred previously.

To obtain detailed information about temperature influence on growth in course of time, which is needed in such a system, growth over short intervals must be measured. Such measurements are a theoretical as well as a technical problem. Technically they are a problem because destructive methods would require enormous numbers of plants to measure growth with any accuracy, whereas continuous measurements on fresh weight are strongly influenced by water status. Measurements of leaf length or plant height may give important information, but still represent only one aspect of growth.

There is a theoretical problem because growth consists of many processes each of which may exhibit a different daily pattern and different temperature reactions. For example dry weight growth or photosynthesis of a plant only takes place during the day, whereas growth in dimensions is faster during the night. Necessarily short-term reaction of growth on temperature, measured in one of the ways mentioned above, will stress only one aspect neglecting others (e.g. Takakura, 1975; Takakura et al., 1975).

Furthermore, after a certain period of exposure to a changed temperature plants show morphological and physiological adaptations (Sawada & Miyachi, 1974; Hussey, 1963), which in their turn will influence plant growth. Such adaptations occur so slowly, that it is highly improbable that these changes are measurable over short intervals.

In the search for optimization of the glasshouse climate one is therefore faced with the following major problems:

- If one considers growth as a permanent change in volume, the effect of a change in environment on plant growth is generally twofold, in that it af-

fects the growth process directly but concomitantly other processes as well. These will influence plant growth with some delay of time. The quantitative relation between these two groups of processes is complex.
- Plant reactions on the environment may depend on plant status, which is the result of the integrated effect of previous and prevailing environmental situations.
- Different growth factors may interact strongly.
- Climate control in glasshouses is rather primitive in that it exists of a heating system and a ventilation system only. As a result, manipulation of one factor usually produces changes in other factors as well, and these changes have to be taken into account.

In spite of large amounts of work on this subject, most of the problems still remain unsolved. Thus the rules for optimum climate control are probably complicated, being the ultimate result of a calculation of the intricate balance between different processes.

With the recent progress in understanding respiration in relation to growth (Penning de Vries, 1974) and the development of explanatory models for the simulation of plant growth (De Wit, 1970), the following strategy was developed:
1. describing and analysing the diurnal course of some important physiological processes simultaneously, under constant environmental conditions, but with a normal day/night rhythm;
2. changing one factor at a time and considering the simultaneous adaption of different processes in course of time;
3. building of a model for the simulation of plant growth under glasshouse conditions, which incorporates the knowledge obtained in phase 1 and 2.

The strategy described here is primaly concerned with the problem of finding a climate control, which under the prevailing conditions will lead to maximum growth over a period of 24 h. Long-term optimization is concerned with a much slower process of morphological and physiological adaptation. This type of optimization requires a different approach, for which the work of Horie et al. (1976) provides a good base. Ultimately both types of approach will have to be combined so that this long-term aspect is taken into account in the diurnal optimization. Simulation models can play an important role in this research because interrelations between different external factors and plant processes can be taken into account and evaluated.

The present work does not include all the aspects mentioned but fits into this strategy. Its first aim is to provide a basis for the development of

a simulation model, in which the internal plant organization is more extensively incorporated than in the present models (De Wit et al., 1970). Nevertheless, the simultaneous study of CO_2 exchange, formation of carbohydrate reserves and transpiration in course of time, in combination with a quantitative analysis of respiration, represent new elements in the study of plant growth so that the information obtained in this study should be valuable from a scientific and a horticultural point of view.

As an experimental plant cucumber was chosen because it is an important glasshouse crop in the winter season and because it has properties that make it suitable for physiological experiments, such as a high growth rate, easily accessible leaves and quickly germinating seeds.

Although an analysis of crop growth under poor light conditions is the primary subject of this study, a comparison with plants growing with an ample supply of light should contribute much tot the understanding of the observed phenomena. Therefore the studies were carried out with two types of plants: 'winter plants', growing under the average January light conditions, and 'spring plants', growing under the average March/April conditions in the Netherlands.

Only one developmental stage, the 5-leaf stage was taken into consideration. At this stage considerable diversity in leaf characteristics is obtained. The use of older plants would have complicated the experiments considerably.

2 Materials and methods

2.1 THE CULTIVATION OF THE PLANTS

For the purpose of this study it was important to grow reproducible plants with small variations in size and growth rate. To reduce these variations to a minimum, plants were grown under controlled environmental conditions in the phytotron of the Centre for Agrobiological Research (CABO).

Genetic variations were excluded by using a F_1 hybrid cucumber, *Cucumis sativus L.* 'Sporu origineel'. Variations in the root environment, which may exert a strong influence on plant growth (Pieters, 1974), were minimized by the use of hydrophonics. The procedure followed to obtain standard plants is described in Sections 3.1 and 3.2.

In the climate rooms used for the cultivation of plants, light was supplied by high pressure mercury lamps (Philips, HPLN 400 W), with a total installed power of ± 3000 $W.m^{-2}$, yielding a maximum visible irradiance (400-700 nm) of ± 90 $W.m^{-2}$. The lamp compartment was separated from the growth room by double glass, to reduce the energy load on the cooling system. 12.5% of the power installed in mercury lamps was additionally installed in incandescent lamps, to increase the proportion of near infrared radiation. This percentage, however, is far from sufficient to obtain a balance between red and infrared radiation, comparable to that of solar radiation (Gaastra, 1970).

This difference may be the cause of the much more compressed appearance of seedlings grown under artificial light compared with those grown in the greenhouse. Extending the 8 h photoperiod of winter plants by 15 min of exclusively incandescent light proved to be sufficient to obtain the 'greenhouse type' of plant. As this effect could be reversed by an extra 15 min of weak red irradiation of TL 15 (Philips), which is poor in infrared, it is quite probable that phytochrome effects are involved. Thus the compressed appearance of the seedlings growing under artificial light may be a result of spectral quality of the light.

The amount of irradiation of the plants was changed by varying the height of the platform of the plant truck on which they were growing (Gaastra, 1968)

and by changing the number of lamps burning.

Irradiation measurements were carried out at the level of the plant with a selenium barrier-layer photocell, made by the Technical and Physical Engineering Research Service (TFDL). The photocell was provided with a neutral grey filter to obtain a linear response up to full sunlight (within 5%). A scattering cap was placed on top for cosine correction. Plant height and the number and distribution of burning lamps were combined in such a way that deviations from the average irradiation were less than 6%.

The photocell, since it had a non-equi-energetic spectral response, was calibrated in situ with the aid of a solarimeter (Kipp and Zonen, Delft, the Netherlands, type CM3) for measurements of visible light energy. With this instrument the amount of visible radiation (400-700 nm) was measured by comparison of the total amount of radiation and the readings obtained after insertion of a RG 695 and a GG 385 filter (Schott[1]) respectively. These filters provide steep wavelength cut-offs near 700 and 400 nm.

Air temperature was kept at 25 ^{o}C ± 0.5 ^{o}C and relative humidity remained within the range of 80-85%. Temperature of the root medium was generally somewhat higher than in the air, as a result of irradiation of the containers and dissipation of heat by a pump, but the difference was always less than 1.5 ^{o}C.

The concentration of carbon dioxide in the air of the climate room was not controlled and thus depended on the outside concentration and on the presence of people in the room. On the average, in the absence of people concentration was in the range of 350-400 $\mu l.l^{-1}$. For these high values no explanation can be given at present. Air movement in the room was horizontal with a wind speed of about 0.2 - 0.4 $m.s^{-1}$.

Because I wanted to investigate the roots apart from their substrate in growth and in respiration experiments, plants were grown on aerated nutrient solution. The macro-element composition was according to Steiner (1968). Per litre solution 5 ml stock solution, containing 131.36 FeNa-EDTA (EDTA = ethylene-diaminetetra-acetate) per 20 l was added. Micro-elements were added according to Table 1. pH of the solution was 6.5 and its osmotic value was 0.7 atm. The water culture in which the plants were grown was described by Steiner (1965). Solution was pumped continuously from a central reservoir to a distribution point from where it was directed to the individual plant pots. The solution fell into the pots and back into the central reservoir. By this

1. JENAer Glaswerk Schott & Gen., Mainz, Germany, (BRD).

Table 1. Composition of micro-element solution according to Steiner (pers. commun.). Of this solution 0.1 ml is added per litre nutrient solution.

$MnSO_4.1\ H_2O$	- 20	g
H_2BO_3	- 27	g
$ZnSO_4.7\ H_2O$	- 5	g
$CuSO_4.5\ H_2O$	- 0.8	g
$Na_2MoO_4.2\ H_2O$	- 1.2	g
Distilled water to 1.0 litre		

action the solution was thoroughly aerated (Steiner, 1968). The level in the pots was kept at a constant level by an overflow.

The system has the advantage that the roots do not become easily entangled and that the solution level remains constant in the plant pots. Furthermore the large common reservoir (± 70 l) assures equal conditions in all pots, whereas changes in the solution are strongly buffered. Total solution volume is about 100 l. pH of the solution was checked weekly and corrected if necessary. At the same time, tap water was added to attain the original level in the central reservoir. Fresh solution was provided every 3 weeks.

The plants grew well and fast, but they were somewhat yellow. Extra application of Mg^{2+} or of FeNa-EDTA did not change this. The hypocotyle exhibited a pronounced bend just below the transition zone between air and solution, for which no explanation could be given.

Plants were positioned so that they did not hinder each other and that side effects in the climate room were avoided. Although the total useful area in the rooms was about 10 m^2, these requirements limited the maximum amount of 5-leaf plants to about 60-70 plants per room.

2.2 MEASUREMENTS OF GAS EXCHANGE

The system used for the measurements of the rate of gas exchange is of the 'open system' type (Jarvis & Čatský, 1971) and consists of the following components:

- Assimilation chamber in which temperature and irradiance are controlled within narrow limits.
- System for the preparation of air mixtures with constant concentrations of CO_2 and water vapour passing at a constant flow rate through the assimilation

chamber.
- Analysing system in which air samples are drawn through the CO_2 and water vapour analysers, for the evaluation of the change in concentrations effected by the organ being studied and for checking the CO_2 concentration in the air prepared in the mixing system.
- 12 point potentiometric mV-recorder with 3 measuring ranges, recording the mV signals provided by gas analysers, thermocouples and photocell.

2.2.1 The gas exchange chamber

Depending on the organ(s) studied, 3 different chambers were used: a single leaf chamber, a whole shoot chamber and a root vessel. For the single leaf measurement a type of chamber, described by Pieters & Schurer (1973) was used, modified slightly by increasing the area 4 times.

It was an unstirred chamber made of Duralumin and provided with double water-cooled windows. The area of the chamber 22.5 x 23 cm (external) was such that only the largest leaves had to be trimmed to fit into it. A slit in the sidewall sealed hermetically by aquarium putty permitted the measurement of leaves attached to the plant.

Temperature of the air was controlled by passing thermostated water through the water jackets of the chamber. Initially the incoming air was passed through a thermostated coil, but this proved to be of little importance in the control of air temperature, due to the small heat capacity of the air. Within the range of irradiance used in this study (usually up to 80 $W.m^{-2}$), the temperature control was satisfactory. Air temperature increased at the highest irradiance to about 1.5 ^{o}C above the wall temperature and leaf temperature remained, depending on its transpiration rate, a few tenths of a degree below the air temperature.

Air temperature was measured with a copper-constantan thermocouple ($d \pm 1.5$ mm) placed below the leaf. Leaf temperature was measured with a thin copper-constantan thermocouple ($d \pm 0.25$ mm) pressed against the lower leaf surface. The reference junctions were put into an electronic reference device made by the TFDL, or in a Dewar flask filled with a mixture of distilled water and ice. The emf produced by the thermocouples was measured on the 1 mV range of a potentiometric recorder.

The errors involved in the measurement of leaf temperature (Pieters & Schurer, 1973) are likely to be small under the conditions described here, because the temperature gradients (see above) between leaf and air and be-

tween air and wall were small due to the moderate irradiation.
As light source a high pressure mercury/iodine arc lamp was used (Philips, HPI/T, 400 W) mounted in a reflector, provided with a mirror extension of 50 cm length. In combination with this extension a very homogeneous irradiance over the leaf surface (maximum variations ± 5%) of maximally about 250 $W.m^{-2}$ (400-700 nm) was obtained.

The voltage of the lamp was kept constant with a magnetic AC voltage stabilizer resulting in a constant light output. Leaf irradiance was varied by inserting neutral filters made of X-ray film sheets of different transmission. The filters were protected against overheating by a double-glass screen.

Irradiance in the chamber was measured continuously with a very small photocell (Siemens, BPY 43) provided with a temperature-insensitive shunt of 10 Ω. The resulting emf was recorded on a 12-point potentiometric recorder.

Calibrations of the photocell were made with a solarimeter (Kipp, CM-3) according to the procedure described in Section 2.1. Extreme care, however, had to be taken to ensure that the spatial light distribution during the calibration was the same as in the measuring situation. Serious mistakes can be made otherwise because of the small opening-angle of this photocell.
Air movement in the chamber, being unstirred, is entirely depended on the rate of air flow. Low flow rates may cause a high boundary layer resistance (r_a) and result in poor leaf temperature control, and poorly defined concentrations of CO_2 and water vapour near the leaf. Furthermore these low flow rates may give rise to steep temperature gradients and high gradients of CO_2 and water vapour concentrations over the leaf in the direction of the air flow (Gaastra, 1959; Jarvis & Čatský, 1971).

Rate of air flow was usually chosen so that CO_2 concentration was decreased only by 20 $\mu l.l^{-1}$ or less at 300 $\mu l.l^{-1}$ and water vapour concentrations increased by about 2 $g.m^{-3}$ (at a concentration of 17.1 $g.m^{-3}$). With small leaves the gradient over the leaf may be somewhat steeper as a result of imperfect mixing within the chamber. Flow rate was usually in the range of 200-600 $l.h^{-1}$, corresponding to a calculated linear rate of air flow of 0.01 - 0.04 $m.s^{-1}$. Turbulence in the chamber, however, was improved by jetting the air through small pipelets into the chamber.

The diffusion resistance in the boundary layer between leaf and air (Gaastra, 1959) has been estimated with an artificial leaf developed by K. Brown and P. Gaastra in this laboratory, but not described elsewhere. Its working principle is based on the determination of the temperature difference

between leaf and air, resulting from the generation of a known amount of heat energy in the leaf by an electric current passing through a resistance wire positioned inside the leaf.

Metal foil with low coefficients of long-wave absorption and emission was chosen for construction of the leaf surface, and the temperature difference between surroundings and leaf was kept small so that convection was the predominant form of heat transfer to the surroundings. From the amount of electric energy input into the artificial leaf in combination with the temperature difference between leaf and air, the value of r_a can be calculated. With a circular artificial leaf (d = 10 cm) values of 1.1-1.3 $s.cm^{-1}$ were found, which is in the range of values normally encountered in assimilation chambers (Jarvis, 1971b).

Changing the rate of air flow during measurements of plant leaves clearly changed the rate of transpiration but did not affect the rate of CO_2 uptake. During measurements of CO_2 uptake, the roots were kept in an aerated, thermostated nutrient solution, which was also used for the cultivation of the plants (Section 2.1). For the environmental control of the remainder of the shoot no special measures were taken. Humidity in the laboratory was increased somewhat by a simple evaporator to avoid excessive transpiration. Part of the shoot received some radiation from the lamp used for the irradiation of the leaf chamber. In the short-term experiments described in this study, it was assumed that CO_2 uptake of the leaf under investigation was not or negligibly influenced by the remainder of the shoot, as long as excessive transpiration could be avoided. The results obtained from single leaf measurements and with whole shoots gave little cause to doubt this point.

CO_2 production of leaves was measured in the same chamber as described above, but as it was expected that the environmental conditions of other plant parts might influence the results, the experiments were always carried out in a climate room. Very low rates of air flow (± 20-50 $l.h^{-1}$) were required to obtain appreciable changes in CO_2 concentration, but the problems mentioned before, which are associated with a low air movement did not play a role in this type of measurement.

For the measurement of gas exchange of complete shoots, a large stirred chamber as described by Louwerse & Van Oorschot (1969) was used. Temperature control was obtained by placing the chamber in a climate room of the CABO phytotron (Section 2.1). The lamps of the climate room were used for irradiation of the chamber. Insertion of a water filter (± 1 cm water) between the lamps and the assimilation chamber proved to be necessary to diminish the

temperature difference between the air in assimilation chamber and climate room, and the associated risk of condensation in the gas handling system. The irradiance in the chamber was measured and the selenium barrier-layer photocell was calibrated under these conditions as described in Section 2.1. Because this type of photocell is somewhat sensitive to infrared radiation, the calibration factor found with water filter was different from that without the filter!

The CO_2 production of the root system was measured in a glass vessel (fig. 1b), partly filled with nutrient solution of the same composition as used for the cultivation of the plants (Section 2.1). About three-quarters of the vessel was immersed in a thermostat bath to control the temperature of the solution. Air was bubbled through the solution and left the vessel through an outlet above it. To avoid condensation in the gas handling system and to protect it from the overflowing solution, a thermostated cooling coil kept at 5 ^{o}C lower than the vessel temperature was put in series with the vessel. The hypocotyle of a plant was passed through a hole in a rubber plug, divided lengthwise into two halves, which was hermetically sealed with plastic aquarium putty.

It proved to be of utmost importance to construct the vessel in such a way, that microbial activity was reduced to a minimum. Use of porous materials thus must be minimized, whereas easy cleaning of all wet parts is essential. In the system used in this study, CO_2 production of the system without the plant root was always below 10% of that of the plant root.

Measurement of CO_2 production in aqueous media is also difficult because CO_2 is highly soluble in water, and its solubility depends on pH and temperature. To minimize these effects, the temperature in the vessel was kept carefully constant and the solution volume was kept small ($\pm$ 0.25 l). Occasional measurements indicated, that the rise in pH after 24 hours was negligible.

Solubility of CO_2 in water, when in equilibrium with the CO_2 of the air (300 $\mu l.l^{-1}$), at atmospheric pressure and 25 ^{o}C is 0.58 mg $CO_2.l^{-1}$ at pH = 6 and 2.12 mg $CO_2.l^{-1}$ at pH = 7. The amount of CO_2 dissolved in the solution thus was small compared to the amount produced by the roots in 24 h: 41.4 mg $CO_2.24\ h^{-1}$ in winter plants and 214 mg $CO_2.24\ h^{-1}$ in spring plants (Section 4.2). Response time of the system was rather slow, as a result of CO_2 exchange with the solution and the low rates of gas flow which were required, but reasonable values could be obtained within 30 min.

2.2.2 *Preparation of gas mixtures with constant concentrations of CO_2 and water vapour*

For the measurement of CO_2 production in a single leaf or in the root system, air of a constant CO_2 concentration was obtained from cylinders (medical quality). Because of the absence of light and the low rate of air flow used in these experiments, no attempt was made to control the air humidity, which thus depended on the transpiration of the leaf and the prevailing rate of air flow. The gas circuit in this set up is shown in Fig. 1a.

In the other cases it was impractical to use cylinders because of the large amounts of air which were required. Therefore a device was developed to mix pure CO_2 with CO_2-free air in the ratio desired (Fig. 2). CO_2 from the air was removed with a selfindicating CO_2-absorber used for medical purposes.

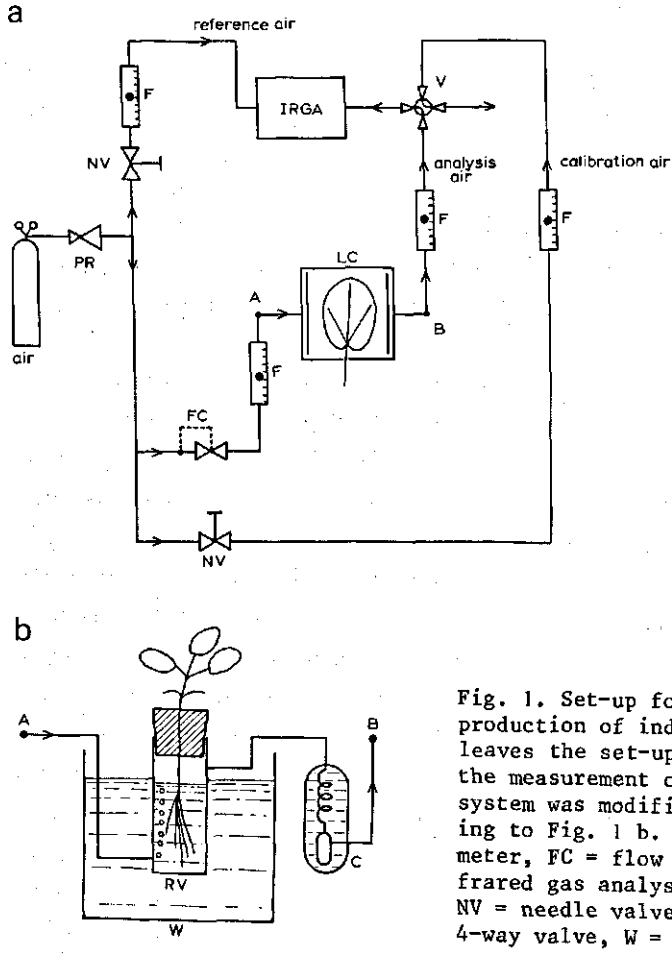

Fig. 1. Set-up for the measurement of CO_2 production of individual plant organs. For leaves the set-up of Fig. 1a was used. For the measurement on the root system the system was modified between A and B according to Fig. 1 b. C = condenser, F = flow meter, FC = flow controller, IRGA = infrared gas analyser, LC = leaf chamber, NV = needle valve, RV = root vessel, V = 4-way valve, W = thermostated water bath.

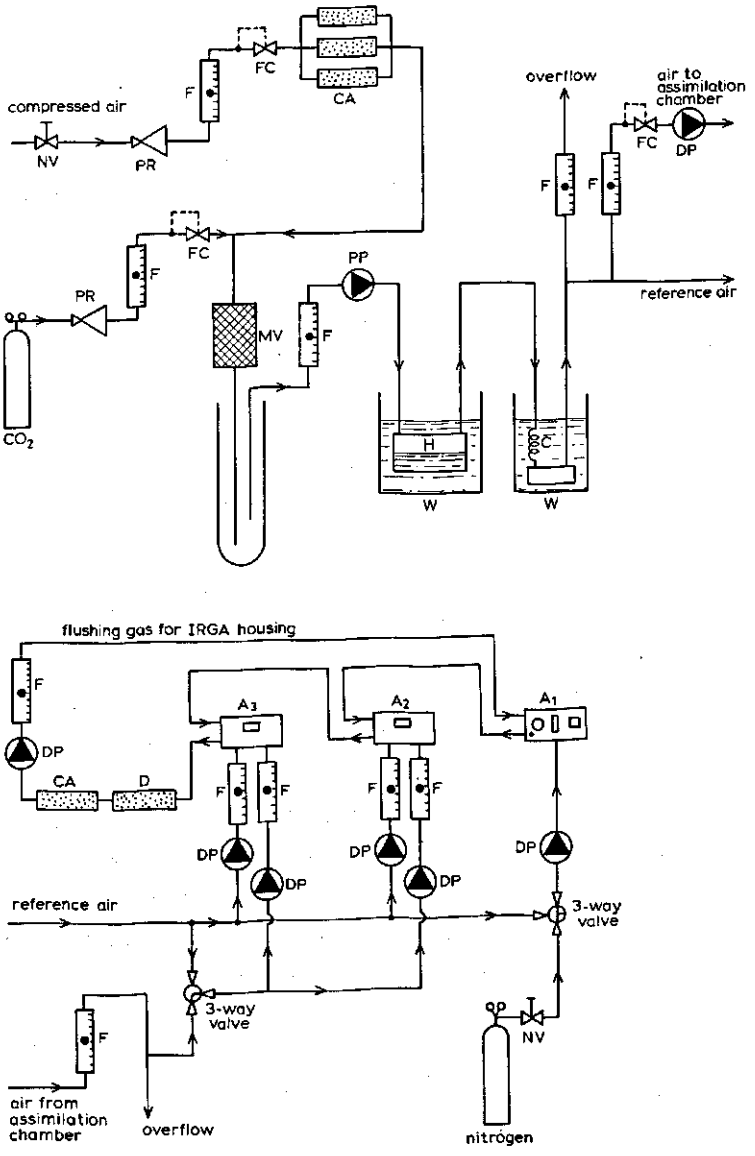

Fig. 2. Set-up for the measurement of CO_2 uptake and the associated gas mixing system. A_1 = analyser URAS 2T measuring CO_2 concentration absolutely, A_2 analyser URAS 2 measuring CO_2 concentration differentially, A_3 = analyser URAS 2 measuring water vapour concentration differentially, C =condenser, CA = CO_2 absorber with soda lime, D = drier with $CaSO_4$, DP = diaphragm pump, F = flow meter, FC = flow controller, H = humidifier, MV = mixing vessel, NV = needle valve, PP = oil free piston pump, PR = pressure regulator.

This absorber was put in three parallel tubes of 4 cm i.d. and 55 cm length. The parallel connection enabled the exchange of one tube at a time without

disturbing a measurement. The tubes were positioned after the flow controller to avoid the danger of the tubes exploding. They were made of glass to show the colour of the CO_2 absorber.

Pure CO_2 was injected in this gas stream at a constant rate of a few tenths of a litre per hour by a combination of a precision pressure controller (Brooks[1], 8601) and precision flow controller (Brooks, 8744) designed for gas chromatography. It proved to be very important to reduce the volume between flow controller and the point of injection to a minimum for obtaining a constant stream of CO_2. Immediately after the point of injection, both streams were mixed thoroughly in a glass tube ($\pm$ 1 dm^3) filled with glass wool.

In a second stage the air mixture was himidified at a constant dewpoint, by passing it over a water surface at high temperature ($\pm$ 7 ^oC above the desired dewpoint) and subsequently through a thermostated cooling coil. The difference between atmospheric pressure and that in the coil was negligible ($\pm$ 2 cm H_2O).

Water vapour and CO_2 concentration of the mixture were constant in time, whereas rapid changes of the CO_2 concentration could be made without long-lasting adaptations. CO_2 concentration of the mixture was continuously measured with an infrared gas analyser (Hartmann & Braun[2], URAS 2 T), and recorded on the 12-point recorder. For the control of the rate of air flow through the assimilation chamber, a flow controller was placed before the pump, which thus was working at a strong underpressure. This was necessary because otherwise condensation would have occurred when the humid air was compressed.

Rate of air flow was measured with rotameters (Brooks), which were calibrated by a flow rate calibrator (Brooks, Vol-u-meter 1057) and a wet precision gas meter.

2.2.3 *The gas analysing system*

The gas analysing system (Fig. 2) consisted of three infrared gas analysers, and the associated gas handling system. One analyser A_1 (Hartmann & Brown, URAS 2 T) measured absolutely CO_2 concentration in the gas mixture which was used for the gas exchange measurements. The other analysers (URAS 2) were operated differentially, measuring the change in CO_2 concentration (A_2)

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1. Brooks Instrument Division, Emerson Electric Co., Veenendaal, the Netherlands.
2. Hartman & Braun, Frankfurt, Germany (BRD).

or water vapour concentration (A_3) of the air mixture caused by the gas exchange in the assimilation chamber.

The absolute analyser was provided with a measuring cuvette of 210 mm length, and was working at a full-scale sensivity of 500 or 1500 $\mu l.l^{-1}$ switched by an electric range selector. Analyser A_2 was provided with a measuring cuvette of 250 mm length and was working at full-scale sensitivity of 30 $\mu l.l^{-1}$. Both analysers were provided with optical filters mounted in the detector side of the measuring cuvette, depressing water vapour sensitivity (Janač et al., 1971). Analyser A_3 had a measuring cuvette of 100 mm length and was working at full-scale sensitivity of 3 $g.m^{-3}$ water vapour content. During the measurements the housing of the analysers was flushed with dry, CO_2-free air, for improvement of their stability. Frequent zero point checks were made by switching a 3 way valve passing reference gas mixture through the analysing cuvette of the differential analysers. Zero point of the absolute analyser was checked by flushing the measuring cuvette with pure nitrogen.

2.2.4 *Calibration of the gas analysers*

CO_2 analysers were calibrated with gas mixing pumps. Fig. 3 shows the calibration set-up which was developed by K. Brown and P. Gaastra at CABO and

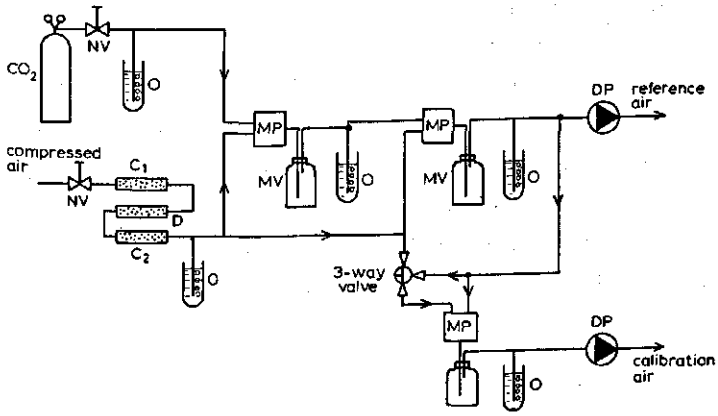

Fig. 3. Calibration system for differential CO_2 analysers with 3 mixing pumps. C_1 = CO_2 absorber with self indicating soda lime, C_2 = CO_2 absorber with soda asbestos, D = drier with $CaSO_4$ (self indicating), DP = diaphragm pump, MP = mixing pump, MV = mixing vessel, NV = needle valve, O = overflow pressure regulator, filled with liquid paraffin.

slightly modified later. It consists of 3 gas mixing pumps (Wösthoff[1], SA27/3a and SA27/2a) put in series. With the first two pumps (SA27/3a) mixtures can be made in the range of 100 - 10,000 $\mu l.l^{-1}$. The third pump (SA27/2a) which has a smaller capacity was used for the calibration of the differentially operated analysers, diluting 90% of the mixture obtained from the first two pumps with 10% CO_2-free air. At 300 $\mu l.l^{-1}$ a difference of 30 $\mu l.l^{-1}$ was obtained, at 400 $\mu l.l^{-1}$ a difference of 40 $\mu l.l^{-1}$, etc.

For obtaining constant gas mixtures rather large mixing vessels were required after each mixing pump. We used empty washing bottles of 600 ml. The disadvantage of the large size of these vessels was the long time required (about 15 min) to obtain a stable CO_2 concentration after a change. After each vessel an overflow-pressure regulator filled with 2 cm liquid paraffin avoids overpressure and contamination with atmospheric air. The short term stability of the concentration difference obtained was better than 1%. For the calibration of differential analysers the system has the advantage over other systems, in which the two concentrations are prepared independently (e.g. calibration cylinders), that mixing errors are not inflated in the concentration difference.

A simple system for the calibration of differential water vapour analysers was developed, based on a principle that to my knowledge has not been described elsewhere in plant physiological literature. In the same thermostat bath in which the condensation spiral (C, Fig. 2) was immersed, a second spiral was placed connected by a sintered glass filter (air resistance) to the supply of the first spiral (Fig. 4).

In the second spiral on underpressure was maintained by drawing air at a constant rate through the glass filter and the spiral. In our set-up this was most easily accomplished with the pump that normally supplies the assimilation chamber with air. Because the temperatures in both spirals were equal, so were the water vapour pressures. After the air from the second spiral returned to atmospheric pressure a different water vapour content resulted. Its value depends on the pressure difference and the waterbath temperature, which can both be measured accurately.

At 760 mm Hg (atmospheric pressure) and 25 ^{o}C (average measuring room temperature),

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1. H. Wösthoff O.H.G. - Bochum, Germany (BRD).

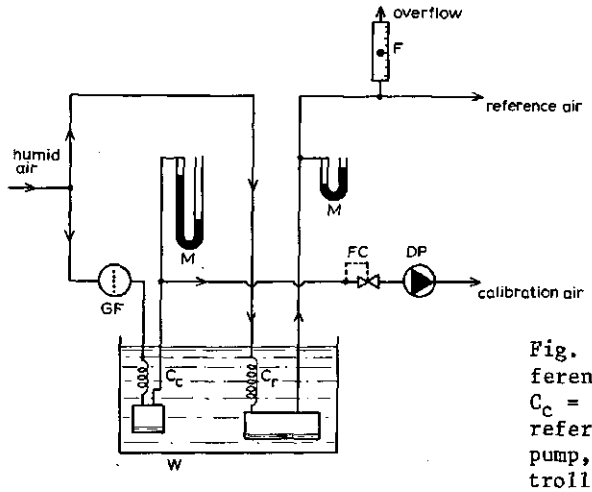

Fig. 4. Calibration system for differential water vapour analysers. C_c = calibration condenser, C_r = reference condenser, DP = diaphragm pump, F = flow meter, FC = flow controller, GF = sintered glass filter, M = manometer, W = thermostated water bath.

$$\chi = 0.97\ e$$

where χ = water vapour content of the air ($g.m^{-3}$)

e = water vapour pressure (mm Hg)

If in the condensation spiral water vapour pressure = e_s, then after getting rid of the underpressure,

$$e' = e_s \frac{P}{P-\Delta P}$$

where e = water vapour pressure at atmospheric pressure

P = absolute pressure in reference spiral

ΔP = pressure difference between condensation spiral and reference spiral.

The difference between the water vapour pressure of the calibration air stream and that of the reference air is

$$e = e' - e = e_s \left(\frac{P}{P-\Delta P} - 1\right) = e_s \frac{\Delta P}{P-\Delta P}$$

and thus at 25 ^{o}C and 760 mm Hg

$$\Delta\chi = 0.97\ e_s \frac{\frac{\Delta P}{P-\Delta P}}{\frac{760}{P}}$$

2.3 CHEMICAL ANALYSIS

2.3.1 Carbohydrate reserves in different plant parts

At 2 or 4 h intervals, 2 plants growing in a climate room were harvested and then separated into leaves, roots and stem, including growing point, hypocotyle and petioles. Each fraction was immediately put into liquid nitrogen and stored at -20 °C until freeze drying. Freeze-dried samples were ground and analysed for their starch and sugar content. Starch was hydrolysed with amyloglucosidase and sucrose with β-fructosidase, and the resulting sugars were determined enzymatically according to the methods described in the booklet Enzymatische Analysen für die Lebensmittelchemie, supplied by Boehringer (Mannheim, Germany, BRD). These methods are based on Bergmeyer (1970). The analyses were carried out by C.R. Vonk and his staff from CABO.

2.3.2 Chemical composition of different plant parts

For the determination of chemical composition of different plant parts, plants were grown under their standard conditions and harvested when the 5-leaf stage was reached. The plants were harvested shortly before the start of the light period to minimize the effects of variations in carbohydrate reserves.

Immediately after harvesting, plants were divided into different fractions and subsequently dried, first at 70 °C, and finally at 105 °C. For the analysis of winter plants 160 plants were harvested and for spring plants 80 plants. The following fractions were analysed: leaf 5, 4, 3, 2, 1, cotyledons, stem (including petioles and growing point), hypocotyle and roots.

In each of these fractions the following determinations were made:

- total amount of nitrogen (N_{tot}), according to method 2.050;
- NO_3^-- nitrogen (N_{NO_3}), with a specific nitrate electrode in aqueous extract;
- ash, according to method 31.012;
- ash alcalinity, according to method 22.026;
- lipids, according to ISO (1975).

The method numbers refer to Official Methods of Analysis of the Association

of Official Analytical Chemists, 12th edition, 1975, Washington D.C. The determinations were done at the Department of Chemistry, CABO. These basic data were further worked out to obtain the fractional weight of different components.

The fraction of organic nitrogen compounds was calculated from the fraction of organic nitrogen, multiplying it by a factor of 5.8 rather than 6.25, which is usually adopted in plant analysis. Infact this latter value is often too high (Penning de Vries & Van Laar, 1976b). The calculation of the organic nitrogen content by subtracting NO_3^--nitrogen from the total nitrogen content neglects the amounts of NH_4^+ and other inorganic N-compounds present in the plant. Mostly this is possible because comparatively small amounts are involved. For the calculation of the mineral content it was assumed that all NO_3^- and organic anions have disappeared in the ash and have been replaced by CO_3^{2-}. The second term eliminates the weight of this carbonate. If besides carbonates oxides are present in important amounts, instead of multiplying by 30, a value somewhere between 30 and 8 (equivalent weight of CO_3^{2-} and O^{2-} respectively should be used. The amount of minerals thus would have been underestimated.

Alkalinity of the ash is caused by CO_3^{2-} (or O^{2-}) replacing organic acids and NO_3^-. When a correction is made for the equivalents of NO_3^-, ash alkalinity represents the equivalent amount of organic acids. Multiplying this by 55 which is an estimate of the equivalent weight of an average mixture of organic acids, the weight of organic acids was calculated. Presence of carbonates will cause an overestimation of the amount of organic acids at the cost of minerals in which fraction they should be included.

The remaining fraction is considered to be carbohydrates. Lignins are also included in this fraction because they were not determined as a result of problems with methods. In the respiration calculation lignins (Ch 5) are estimated as 10% of this fraction.

2.4 MEASUREMENTS OF INCREASE IN FRESH WEIGHT

It is difficult to measure fresh weight of plants with a high reproducibility because after removal from the nutrient solution the root still contains some solution that gradually drains from it. Moreover water is lost from the plant by transpiration. For accurate measurements of increase in fresh weight over small time intervals, a well defined standard procedure has to be followed.

The following method appeared to give reasonable results: a plant was removed from the solution on which it was growing, and quickly taken to a weighing room. The roots were slightly pressed on filter paper on both sides and immediately placed in a beaker on a balance. After weighing, the plant was removed and the increase of weight of the beaker due to wetting subtracted from the plant weight. The plant was immediately returned to the growing room. A drawback of the system is the handling of the plant which probably affects its growth (Section 4.5).

2.5 GROWTH ANALYSIS

For the determination of plant characteristics, plants growing under standard conditions in the CABO phytotron were periodically harvested always at the end of the night, just before the lights went on.

Usually plants were growing on more than one plant truck and then equal amounts were taken from each. The influence of plant position was eliminated by taking plants at random from the truck.

Plants were dissected into different leaves, roots, hypocotyle and stem, including petioles and growing point. Immediately the fresh weight of each fraction was determined. Next the area of the leaves was measured with an electronic area meter constructed by TFDL. Subsequently the plant parts were dried at 105 ^{o}C and weighed on an analytical balance.

2.6 MEASUREMENTS OF VARIATIONS IN LEAF WATER CONTENT

Variations in leaf water content were monitored with a β-gauge (Nakayama & Ehler, 1964). A 10 μCi ^{99}Tc β-source mounted on top of a small perspex rod was provided by the Institute for Atomic Sciences in Agriculture (ITAL). Transmission of β radiation through the leaf under investigation was measured with a GM tube positioned under the leaf. Count rate was continuously recorded using a rate meter operated at a maximum time constant of 100 s. Simultaneously at 10 min intervals a printed output was provided by a printer/timer . Changes in the water status of the plant due to internal phenomena, were obscured by oscillations caused by the air conditioning of the climate room. Integrated count rates over 1 h had to be considered to get rid of these interferences. However various other factors also may contribute significantly to variations in count rate of this magnitude which makes a quantitave approach difficult (Challa & Hopmans, 1975).

3 Standard spring and winter plants

In this chapter some important characteristics of standard spring and winter plants are described. Measurements were generally made on different plants at different moments and as a result plants were not exactly equal. The concept of standard plant was introduced to compare these different measurements. Thus individual measurements could be incorporated into a whole-plant picture.

Standard plants are plants with 5 unfolded leaves. Their dry weight was chosen rather arbitrarily from a dry weight growth curve and subsequently the corresponding plant characteristics were determined from successive harvest experiments. The data given in this description all refer to plants at the end of the dark period, just before the lights were switched on. Leaves were numbered from old to young.

The conditions under which spring and winter plants were grown were chosen in such a way that daylength and total amount of visible light energy received per day corresponded to the average situation in the Netherlands in March-April and January, respectively.

3.1 DESCRIPTION AND DEFINITION OF THE STANDARD WINTER PLANT

In Table 2 a scheme is presented for the cultivation of standard winter plants. A 5-leaf winter plant weighs about 700 mg and its relative growth rate (RGR) is 0.157 $g.g^{-1}.24\ h^{-1}$. The standard winter plant was defined as a

Table 2. Cultivation of winter plants.

Treatment	Day No	Conditions
sown in darkness	0	28 °C, darkness, seeds in humid perlite
seedlings placed in light	3	phytotron: 25 °C day and night, -2 daylength 8 h, irradiance 30 $W.m^{-2}$, relative air humidity 80%, CO_2 concentration not controlled, 350-400 $\mu l.l^{-1}$.
seedlings transplanted on nutrient solution	4	
standard plant	26	

plant reared according to Table 2 and having a dry weight of exactly 700 mg. Fig. 5 shows dry weight of a winter plant in relation to its age. From this figure it may be concluded that the standard winter plant is growing exponentially, and its growth rate may be calculated accordingly at 119 mg.24 h^{-1}. Leaf area of the standard winter plant was 4.0 dm^2 as may be calculated by linear interpolation between two harvests of the growth experiments of Fig. 5. This procedure gives a good estimate because the ratio of leaf area to dry weight changes only very gradually. For a further characterization of the standard winter plant, the relation between plant dry weight and dry weight and leaf area of different organs was investigated (Fig. 6). Over periods as short as 5 days this relation is approximately linear so that standard plant characteristics can be obtained by linear interpolation (Table 3). The linear relation between organ dry weight and whole plant weight also implies that organ growth during this interval is a constant fraction of whole plant growth. Multiplication of this fraction by total growth rate of the standard plant resulted in good estimates of the growth rate of individual organs (Table 3).

Standard winter plants were young plants in which almost all organs were growing: only the cotyledons were practically full-grown. Fig. 7 shows the relation between leaf number and relative growth rate in dry weight and area of that leaf. For dry weight a linear relation was found for all leaves but

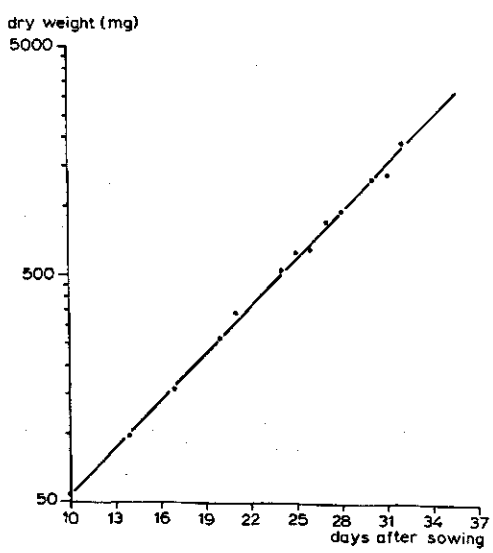

Fig. 5. Dry weight of winter plants against time.

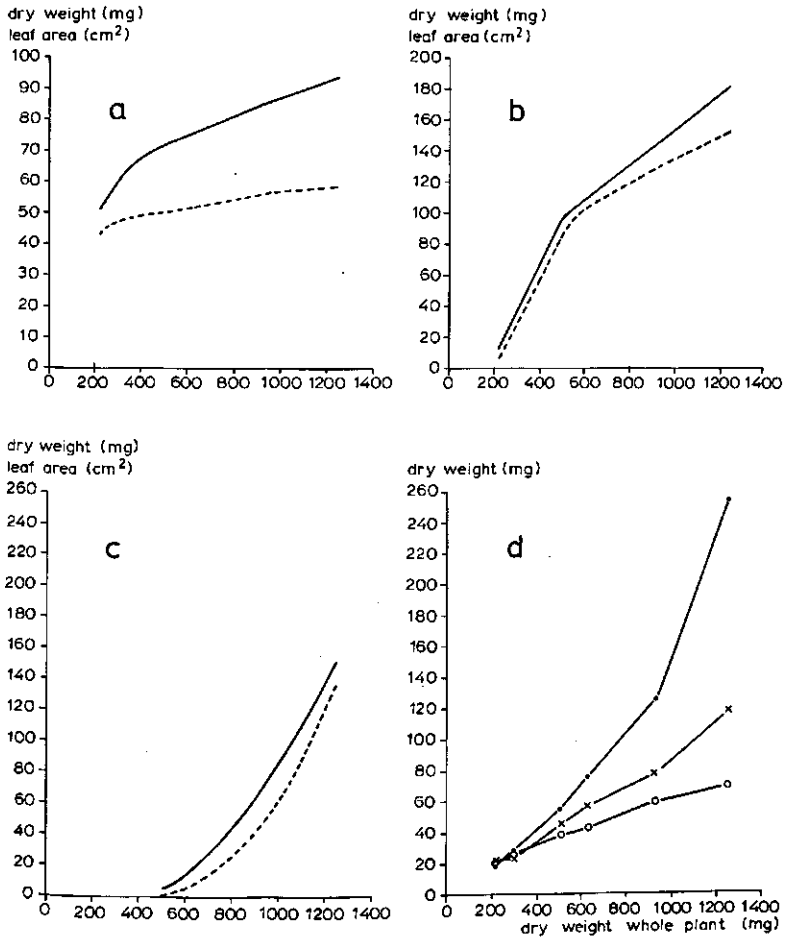

Fig. 6. Relation in winter plants between total plant dry weight and leaf area and dry weight of different organs. a. leaf 1, b. leaf 3, c. leaf 5, d. stem, root and hypocotyle,—— = dry weight, ---- = leaf area, x—x = root, •—• = stem, o—o = hypocotyle.

in the oldest and the youngest leaf some deviation was observed. Fig. 7 also shows that leaf 1 has almost completed its area growth, whereas its weight is still increasing in correlation with the other leaves (Horie et al., 1976).

3.2 DESCRIPTION AND DEFINITION OF THE STANDARD SPRING PLANT

In Table 4 a scheme is presented for the cultivation of standard spring plants. A 5-leaf spring plant weighs about 3000 mg and its relative growth rate is 0.333 $g.g^{-1}.24\ h^{-1}$. The standard spring plant was defined as a plant reared as described in Table 4 and with a dry weight of exactly 3000 mg.

Table 3. Dry weight, leaf area and growth rate of different organs of standard winter plants.

Organ	Dry weight (mg)	Increase in dry weight (mg.24 h^{-1})	Leaf area (dm^2)
roots	57.6	9.92	
hypocotyle	59.9	6.05	
stem[1]	105.4	31.03	
cotyledons	43.3	0.32	0.21
leaf 1	77.2	3.64	0.59
leaf 2	130.3	10.96	1.16
leaf 3	123.8	16.21	1.15
leaf 4	74.2	22.51	0.61
leaf 5	31.0	18.25	0.22

1. Petioles and growing point are included in the stem fraction.

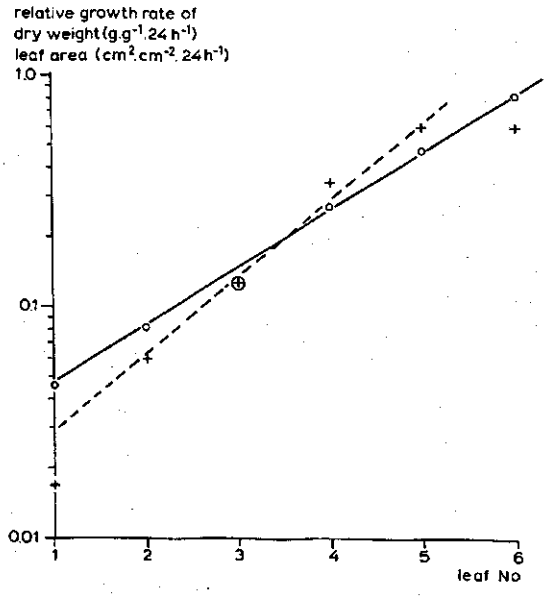

Fig. 7. Relation between leaf number and relative growth rate in dry weight and area in winter plants. o—o = dry weight, +—+ = leaf area.

Table 4. Cultivation of spring plants.

Treatment	Day No	Conditions
sown in darkness	0	28 °C, darkness, seeds in humid perlite
seedlings placed in light	3	phytotron: 25 °C day and night, daylength 14 h, irradiance 80 $W.m^{-2}$, relative air humidity 80% RH, CO_2 concentration not controlled, 350-400 $\mu l.l^{-1}$.
seedlings transplanted on nutrient solution	4	
standard plant	18	

Fig. 8 shows dry weight of a spring plant in relation to its age. Until a dry weight of 4000 mg has been reached, relative growth rate remains constant. Relative growth rate then decreases as a result of strong mutual shading of leaves. Standard spring plants are still in the steep part of the growth curve and have a growth rate of 1185 mg.24 h^{-1}. For the further characterization of the standard spring plant the same procedure as for winter plants was followed. The results are summarized in Table 5, whereas the relations between plant dry weight and the dry weight and leaf area of the organs are presented in Fig. 9. Because spring plants have a much higher relative growth rate than winter plants, the interval for which the linear relationship between plant dry weight and organ characteristics holds is smaller, but nevertheless sufficient for these calculations. Total leaf area of standard spring plants was 8.25 dm^2.

Like winter plants standard spring plants were young plants in which all parts were growing. A plot of the logarithm of relative growth rate of dry weight and leaf area of successive leaves against leaf number gives a linear relation in number 2, 3 and 4 (Fig. 10). Leaf 1 is almost full-grown and de-

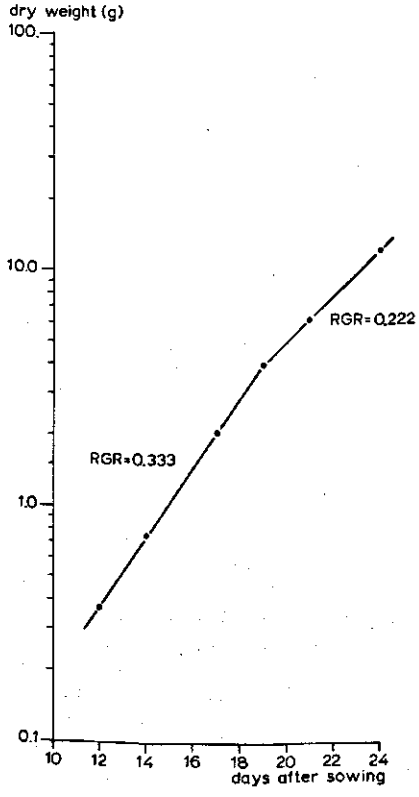

Fig. 8. Dry weight of spring plants against time. RGR = relative growth rate ($g.g^{-1}.24\ h^{-1}$).

Table 5. Dry weight, leaf area and growth rate of different organs of standard spring plants.

Organ	Dry weight (mg)	Increase in dry weight (mg.24 h^{-1})	Leaf area (dm^2)
roots	474	196	
hypocotyle	129	41.3	
stem[1]	341	200	
cotyledons	94.2	7.7	0.245
leaf 1	412	51.4	1.34
leaf 2	646	166	2.68
leaf 3	531	235	2.36
leaf 4	285	209	1.22
leaf 5	88.1	83.3	0.328

1. Petioles and growing point are included in the stem fraction.

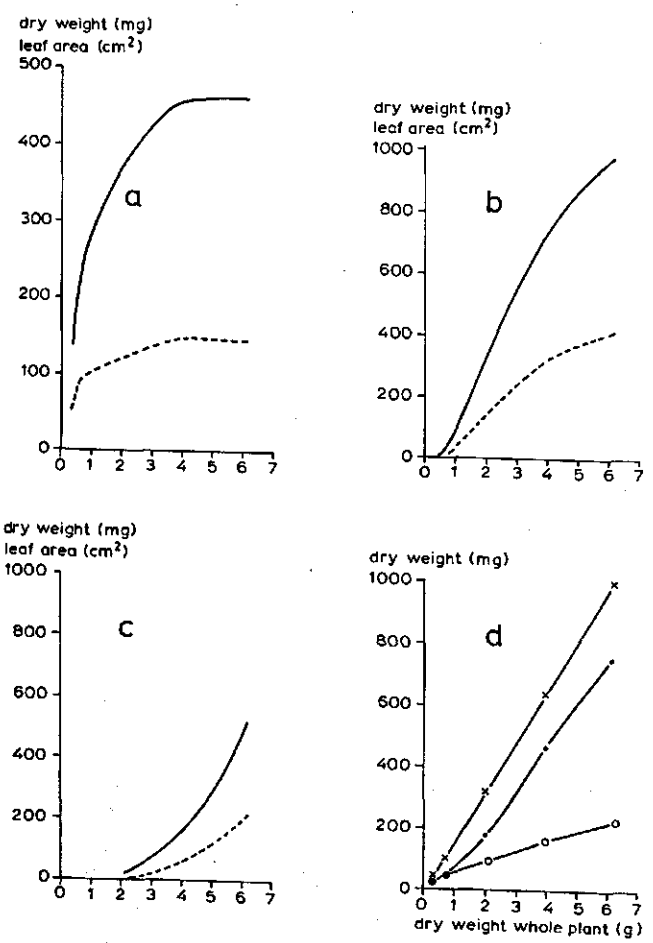

Fig. 9. Relation between total plant dry weight and leaf area and weight of different organs in spring plants. a. leaf 1, b. leaf 3, c. leaf 5, d. stem (.—.), root (x—x) and hypocotyle (o—o). —— = dry weight, --- = leaf area.

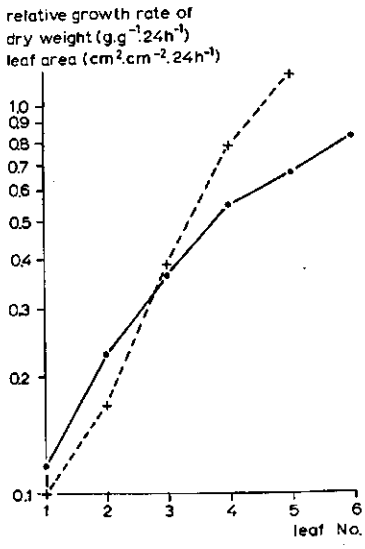

Fig. 10. Relation between leaf No. and relative growth rates for leaf area and dry weight in spring plants.—— = dry weight, --- = leaf area.

viates clearly from this line. The youngest leaves have relative growth rates which, when plotted on a logarithmic scale also exhibit a linear relation to leaf number, buth with a smaller slope. Possibly this is related to their nutritional status, because leaf 4 is in a transient position between autonomous and importing leaves. Horie et al. (1976) found similar bends.

3.3 A COMPARISON OF STANDARD SPRING AND WINTER PLANTS

Standard spring and winter plants both have 5 unfolded leaves, but otherwise they show characteristic differences summarized in Table 6. Spring plants were exposed to an irradiance of 80 $W.M^{-2}$ for 14 h corresponding to a day sum of 1120 $W.h.m.^{-2}$. Winter plants in an 8 h light period were exposed to an irradiance of 30 $W.m^{-2}$, corresponding to a day sum of 240 $W.h.m^{-2}$. Thus spring plants received 5 times more energy per day per unit leaf area than winter plants. In both types of plants about 70% of total dry weight was in the leaves, but winter plants were much more efficient in making leaf area, needing only 123 mg to make 1 dm^2, whereas spring plants for the same leaf area needed 252 mg dry weight. These facts may explain why relative growth rates differed only by a factor two.

Another striking difference between spring and winter plants was the fraction of dry weight in the roots, being almost twice as high in spring plants.

Table 6. Comparison of some important plant characteristics of standard spring and winter plants.

	Spring plants		Winter plants	
dry weight (a)	3000	mg	700	mg
leaf area (b)	8.25	dm^2	4	dm^2
leaf weight (c)	2077	mg	490	mg
root weight (d)	474	mg	57.6	mg
LAR (b/a)[1]	2.75	$dm^{-2}.g^{-1}$	5.71	$dm^{-2}.g^{-1}$
LWR (c/a)[2]	0.69	$g.g^{-1}$	0.70	$g.g^{-1}$
RWR (d/a)[3]	0.158	$g.g^{-1}$	0.082	$g.g^{-1}$
SLA (b/c)[4]	3.97	$dm^2.g^{-1}$	8.16	$dm^2.g^{-1}$
RGR[5]	0.333	$g.g^{-1}.24\ h^{-1}$	0.157	$g.g^{-1}.24\ h^{-1}$
NAR[6]	122	$mg.dm^{-2}.24\ h^{-1}$	27.5	$mg.dm^{-2}.24\ h^{-1}$

1. LAR = leaf area ratio
2. LWR = leaf weight ratio
3. RWR = root weight ratio
4. SLA = specific leaf area
5. RGR = relative growth rate
6. NAR = net assimilation rate, estimated according to the method described in Section 5.5.

3.4 CHEMICAL COMPOSITION OF STANDARD PLANTS AND AN ESTIMATION OF THE COMPOSITION OF DRY WEIGHT ACCRETION

Chemical composition of summer and winter plants was established according to the methods described in Section 2.3.2. The results of these analyses are presented in Table 7. For the calculations in Chapter 5, chemical composition of new growth (referred to as accretion), rather than that of the whole plant has to be known. This information may be obtained by carrying out a second set of analyses on plants, which are one or a few days older. Of course the amount of work involved in such an analysis is considerable, whereas small differences in composition have relatively little affect on the results of the respiration calculations of Chapter 5. Furthermore the accumulated effect of statistical errors of two chemical analyses in addition to the errors in the calculation of growth of different organs over the corresponding interval, have also to be taken into account (Van Egmond, 1975).

A reasonable alternative approximation is obtained by assuming that the change in chemical composition of leaves in course of time is at least reflected in the relation between leaf number and composition, and that other organs have a chemical composition that changes only slowly with time.

Fig. 11 shows the relation between leaf number and the content of various substances. In both spring and winter plants, older leaves show an accu-

Table 7. Chemical composition of dry weight of different organs of 5-leaf spring and winter plants (content given in $g.g^{-1}$).

Organ	Organic N-compounds	Organic acids	Lipids	Minerals	Carbohydrates[3]
Spring plants					
roots	0.182	0.0616	0.0272	0.330	0.399
hypocotyle	0.144	0.0600	0.0294	0.267	0.500
stem[1]	0.211	0.0891	0.0252	0.286	0.389
cotyledons	0.160	0.401	0.0268	0.281	0.131
leaf 1	0.280	0.295	0.0334	0.229	0.163
leaf 2	0.340	0.204	0.0372	0.194	0.255
leaf 3	0.353	0.134	0.0418	0.152	0.319
leaf 4	0.410	0.0869	0.0577	0.110	0.335
leaf 5[2]	(0.47)	(0.07)	0.0538	(0.10)	0.306
Winter plants					
roots	0.262	0.066	0.0245	0.234	0.414
hypocotyle	0.0757	0.0154	0.0060	0.339	0.564
stem[1]	0.169	0.0809	0.0117	0.327	0.411
cotyledons	0.153	0.268	0.0163	0.314	0.249
leaf 1	0.259	0.257	0.0261	0.292	0.166
leaf 2	0.302	0.201	0.0447	0.261	0.191
leaf 3	0.335	0.180	0.0448	0.206	0.234
leaf 4	0.371	0.141	0.0596	0.172	0.256
leaf 5[2]	0.480	0.0825	(0.06)	0.103	(0.275)

1. Petioles are included in the stem fraction.
2. Estimates are given in brackets.
3. The fraction which remains after subtracting minerals, organic N-compounds and organic acids has been interpreted as carbohydrates, though small amounts of other substances will be present in it.

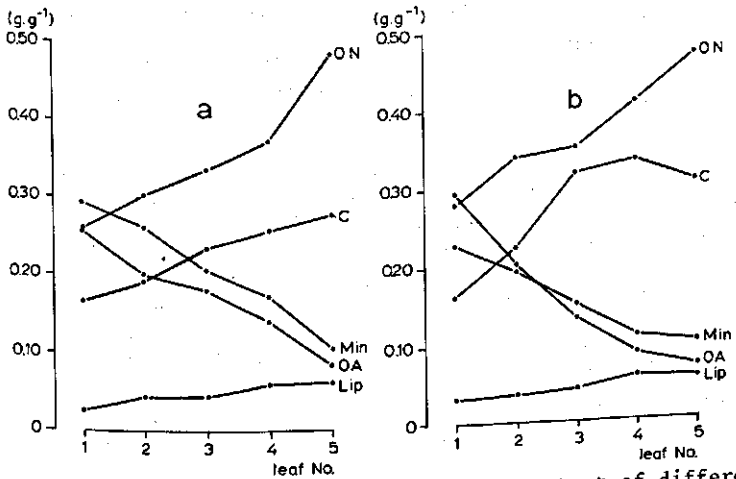

Fig. 11. Relation between leaf No. and content of different plant components in the dry weight of spring and winter plants. a. winter plants, b. spring plants. ON = organic nitrogen compounds, C = carbohydrates, Min = minerals, OA = organic acids, Lip = lipids.

mulation of organic acids and minerals at the expense of the other substances. This observation raises the question whether perhaps shifts in chemical composition in relation to leaf number (age) are the result of a 'dilution' of a certain amount of leaf growth of constant composition by increasing amounts of organic acids and minerals.

Table 8 gives the contents of protein, fat and carbohydrate in dry weight free of organic acids and minerals. It shows that the composition of dry mass free of minerals and organic acids is essentially constant in different leaves, and thus to find the relation between leaf number and chemical composition it is only necessary to estimate the accumulation of minerals and organic acids in leaves in the course of time.

The accumulation of organic acids in leaves may be estimated because an intimate relationship exists between NO_3^- reduction and organic acid formation (Section 5.1), although some assumptions have to be made:

- protein content of the plant remains unchanged;
- all NO_3^- is reduced in the leaves;
- NO_3^- reduction is proportional to transpiration (passive transport of NO_3^-) and hence approximately to leaf area;
- from all organic acids formed in a leaf, 33% is exported in spring plants, and 27% in winter plants (as concluded from a comparison of total amount of organic nitrogen in the plant and the amount of carboxylate that is still in the leaves);
- each leaf exports the same proportion of organic acids as formed in it.

On the basis of these assumptions, increase of organic acid content in different leaves of standard spring and winter plants was calculated (Table 9). All leaves of winter plants and the 3 youngest leaves of spring plants had a

Table 8. Protein, lipid and carbohydrate content ($g.g^{-1}$) in the dry weight of successive leaves, free of minerals and organic acids.

Leaf No.	Winter plants			Spring plants		
	proteins	lipids	carbohydrate	proteins	lipids	carbohydrate
1	0.57	0.058	0.37	0.59	0.070	0.34
2	0.56	0.083	0.36	0.57	0.062	0.37
3	0.55	0.073	0.38	0.49	0.059	0.45
4	0.54	0.087	0.37	0.51	0.072	0.42
5	0.59	0.074	0.34	0.57	0.065	0.37
average	0.56	0.075	0.36	0.55	0.066	0.39

Table 9. Calculated increase of organic acids in leaves of standard spring and winter plants.

Leaf No.	Spring plants		Winter plants	
	Amount accumulated ($mg.leaf^{-1}.24h^{-1}$)	Content in dry weight accretion ($mg.mg^{-1}.24h^{-1}$)	Amount accumulated ($mg.leaf^{-1}.24h^{-1}$)	Content in dry weight accretion ($mg.mg^{-1}.24h^{-1}$)
1	25.5	0.495	2.26	0.620
2	50.9	0.306	4.44	0.405
3	44.8	0.191	4.40	0.271
4	33.2	0.111	2.33	0.104
5	6.2	0.075	0.84	0.046

ratio of 1.2 mg minerals to 1 mg organic acids. Only leaves 1 and 2, and the cotyledons of spring plants showed a different pattern in that for each mg of organic acid in excess of 13% of the dry weight there was 0.48 mg minerals present (Fig. 12). With these relations the chemical composition of the dry weight accretion of different organs can now be estimated (Table 10).

Table 10. Estimated chemical composition of the dry weight accretion of different organs of standard spring and winter plants. Contents are expressed in $g.g^{-1}$.

Organ	Organic N-compounds	Organic acids	Lipids	Minerals	Carbohydrates
Winter plant					
roots	0.262	0.066	0.0245	0.234	0.414
hypocotyle	0.0757	0.0154	0.0060	0.339	0.564
stem	0.169	0.0809	0.0117	0.327	0.411
cotyledons	-	0.45	-	0.55	-
leaf 1	-	0.45	-	0.55	-
leaf 2	0.061	0.41	0.008	0.49	0.039
leaf 3	0.23	0.27	0.030	0.32	0.15
leaf 4	0.44	0.10	0.059	0.12	0.28
leaf 5	0.50	0.046	0.067	0.055	0.32
Spring plant					
roots	0.182	0.0616	0.0272	0.330	0.399
hypocotyle	0.144	0.0600	0.0294	0.267	0.500
stem	0.211	0.0891	0.0252	0.286	0.389
cotyledons	-	0.68	-	0.32	-
leaf 1	0.099	0.49	0.012	0.33	0.070
leaf 2	0.25	0.31	0.030	0.24	0.18
leaf 3	0.35	0.19	0.042	0.18	0.25
leaf 4	0.42	0.11	0.050	0.13	0.30
leaf 5	0.46	0.075	0.055	0.09	0.33

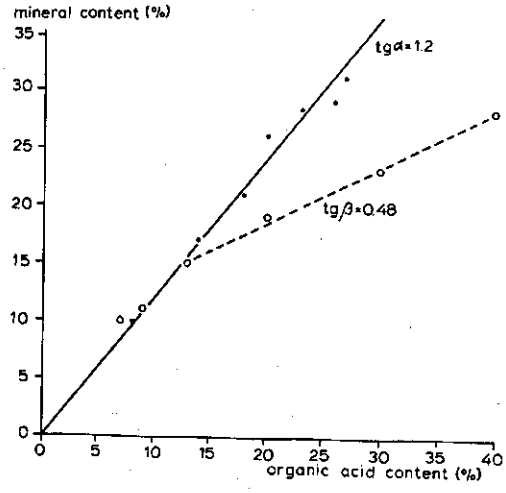

Fig. 12. Relation between organic acid and mineral content in the dry weight of different leaves of spring (o) and winter plants (.).

3.5 LIGHT ABSORPTION BY LEAVES OF THE STANDARD PLANT

The coefficient of light absorption is an important leaf characteristic. It is one of the factors determining the efficiency of leaves and of canopies in utilization of light energy (De Wit, 1965). Therefore from both spring and winter plants leaves 1, 2 and 4 were examined. Differences between leaves 2 and 4 were so small that leaf 3 was not measured, whereas leaf 5 was too small for the equipment used.

The measurements were carried out in the optical laboratory of TFDL by K. Schurer, using a sphere of Ulbricht in combination with a spectrophotometer. First the reflection coefficient was measured. Reflection of the leaf was compared at different wavelengths (400-700 nm, 20 nm steps) with reflection of the innerwall of the sphere. Secondly an external light source was used for measurements of transmission coefficient at different wavelengths. Absorption coefficient for each wavelength interval was calculated according to $\alpha = 1 - (\tau + \rho)$, where α is the absorption coefficient, τ is the transmission coefficient and ρ is the reflection coefficient. Fig. 13 shows the results of these measurements which strongly resemble each other, leaf 1 of spring plants being relatively dark and leaf 4 of winter plants being relatively light.

HPLN lamps were used for growing plants, whereas in photosynthesis experiments HPI/T light was used (Chapter 2). By comparing the spectral dis-

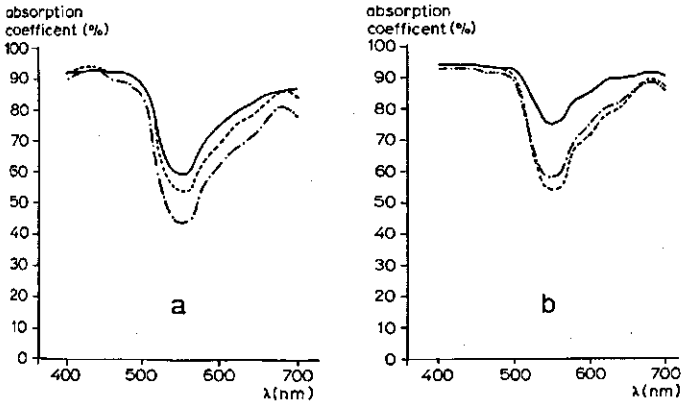

Fig. 13. Absorption spectrum between 400 and 700 nm of different leaves of spring (a) and winter plants (b).

Table 11. Light absorption coefficient of spring and winter plant leaves under HPLN and HPI/T light expressed in %.

Lamp type	Leaf No					
	Spring plants			Winter plants		
	1	2	4	1	2	4
HPLN	86	76	74	76	73	66
HPI/T	90	81	80	81	78	72

tribution of these lamp types with the absorption coefficient at different wavelengths of the leaves examined, light absorption in each combination could be calculated (Table 11). A systematic difference between HPI/T and HPLN light absorption of 7% was found in all cases, HPI/T light giving always a higher absorption.

4 Diurnal courses

This chapter describes the time course of some important plant processes in standard spring and winter plants, growing under their standard conditions. These processes are mainly measured on individual organs. This approach causes some problems when the individual measurements have to be incorporated into a whole plant picture. Still it has many advantages: for single leaves measuring conditions are far better defined than for a whole shoot, and knowledge on the organ level may contribute considerably to the interpretation of the observed relations. This insight is strongly needed because the ultimate purpose of this study is gaining knowledge about plants growing in a greenhouse, under a large range of environmental conditions.

The problem of comparing measurements on individual organs, and thus, for technical reasons, on different plants, has largely been solved by the introduction of the concept of the standard plant (Chapter 3). When measurements have to be compared, results will be expressed on a standard plant basis. Individual leaf measurements are compared with a few whole shoot measurements to evaluate systematic errors possibly introduced by the experimental technique.

4.1 CO_2 UPTAKE OF INDIVIDUAL LEAVES AND OF WHOLE SHOOTS

In the following section the time course of the rate of CO_2 uptake by different leaves will be described. The plant conditions under which the measurements were carried out were not quite standard. With these measurements the CO_2 uptake by different leaves of the standard plant was estimated according to the procedure described here.

In the estimation three factors play a role: irradiance, CO_2 concentration and leaf area. Irradiance and CO_2 concentration were treated as if they were acting independently. The resulting error was quite small because relatively small deviations had to be taken into account. The data of Figs. 14 and 15 were used to evaluate these effects, by calculating the ratio of the rate of CO_2 uptake under standard conditions to that under experimental con-

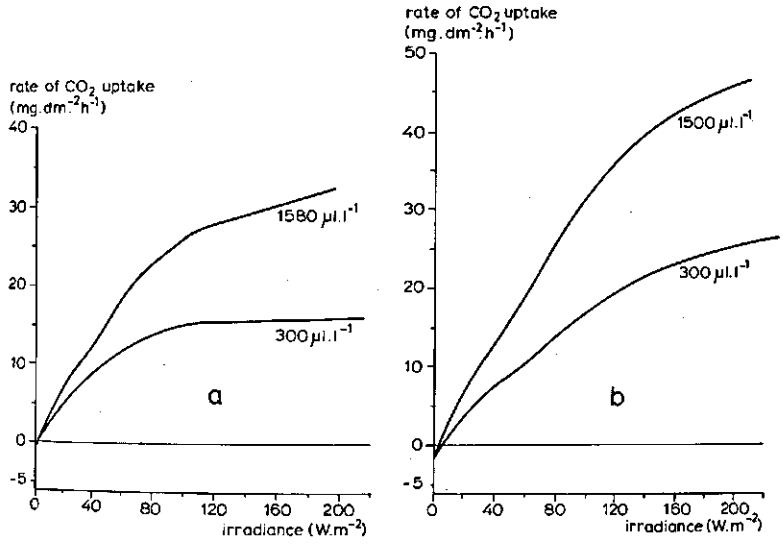

Fig. 14. Rate of CO_2 uptake of leaf 3 of 5-leaf spring (b) and winter plants (a) in relation to irradiance at high and low CO_2 concentration.

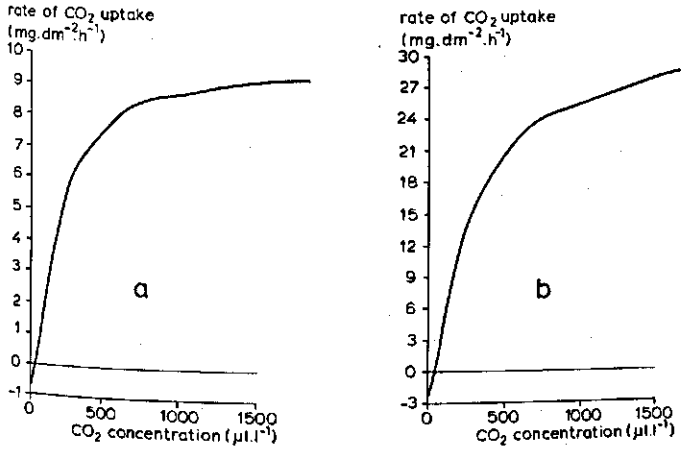

Fig. 15. Rate of CO_2 uptake of leaf 3 of 5-leaf spring (b) and winter plants (a) in relation to CO_2 concentration in the air, at standard irradiance.

ditions. Because CO_2 concentration under standard conditions was not controlled, a value of 375 µl.l^{-1} was assumed to approximate the average concentration. This value was in fair agreement with measurements in which the concentration was in the range of 350-400 µl.l^{-1}.

The rate of CO_2 uptake was expressed on a leaf area basis, but because leaf area was determined at the end of the experiment, it cannot be compared with the leaf areas of standard plants which were determined at the start of

the day. Especially in young leaves relative area growth rate was so high that this effect could not be neglected. Thus for the calculation of CO_2 uptake of standard plant leaves, growth was taken into account.

4.1.1 Daily course of the rate of CO_2 uptake in the leaves of winter plants

Winter plants were growing under low irradiance and a short day (8 h) to approximate conditions in January as far as was possible in a phytotron. The average irradiance over a day in that month, 30 $W.m^{-2}$, is well below the saturating irradiance in these leaves (Fig. 14).

When leaf 3 of a winter plant was exposed to an irradiance of 30 $W.m^{-2}$ for a period of 8 h, little change in the rate of CO_2 uptake was found once the leaf was adapted to the light, i.e. after about one hour (Fig. 16). In some measurements a gradual decrease of CO_2 uptake was noticed about 2 h before the end of the light period. This decrease was accompained with a reduction in transpiration rate (Section 5.7). The rate of CO_2 uptake by a whole shoot essentially showed the same pattern as in leaf 3 (Fig. 16). Thus no further attempts were made to establish this pattern in other leaves.

Values of the rate of CO_2 uptake of different leaves after adaptation to the light are presented in Table 12, together with the average rate of CO_2 production in the dark and the calculated rate of gross photosynthesis. The same table gives the daily amount of CO_2 taken up by the leaves of the stan-

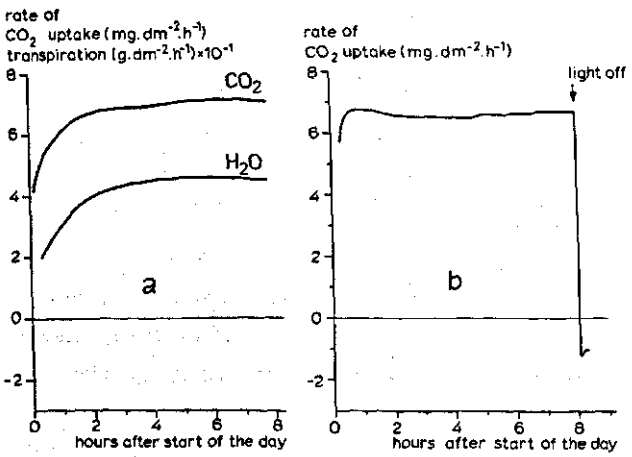

Fig. 16. Daily course of the rate of CO_2 uptake and of transpiration of leaf 3 (a) and of a complete shoot (b) of 5-leaf winter plants under standard conditions.

Table 12. Rate of CO_2 exchange, calculated rate of 'gross photosynthesis' of different leaves of 5-leaf winter plants, measured at 25 °C, 300 $\mu l.l^{-1}$ CO_2 concentration and 37 $W.m^{-2}$ irradiance, and the daily amount of CO_2 uptake by standard plant leaves calculated from the first column.

Leaf No.	a CO_2 uptake ($mg.dm^{-2}.h^{-1}$)	b CO_2 production ($mg.dm^{-2}.h^{-1}$)	a+b 'Gross photosynthesis' ($mg.dm^{-2}.h^{-1}$)	CO_2 uptake of standard plant leaves ($mg.leaf^{-1}.8h^{-1}$)
cotyledons	6.94	0.50	7.44	10.7
1	8.92	0.45	9.37	38.7
2	9.02	0.46	9.48	77.0
3	8.95	0.64	9.59	75.8
4	7.71	0.89	8.60	34.6
5	3.32	3.05	6.37	5.4

dard plant, calculated according to the procedure described in the introduction of this section.

The amount of CO_2 taken up by the whole shoot of standard plants was thus estimated at 242 mg $CO_2.plant^{-1}.8\ h^{-1}$. This value was diminished somewhat by stem respiration, which was about 9.6 mg $CO_2.plant^{-1}.8\ h^{-1}$. Hence an estimate of 233 mg $CO_2.plant^{-1}.8\ h^{-1}$ was obtained.

Measurements on whole shoots showed more variation than single leaf measurements, probably as a result of the less well-defined irradiance per leaf. The measurements were converted to standard plant values. The average of 6 measurements was 218 mg $CO_2.plant^{-1}.8\ h^{-1}$, which compares well with the estimate obtained from single leaf measurements.

4.1.2 Daily course of the rate of CO_2 uptake in the leaves of spring plants

Spring plants were growing under an irradiance of 80 $W.m^{-2}$ and a rather long day (14 h). Daily sum of radiation approximated the average circumstances at the end of March, the daylength corresponding to that of April. This irradiance was in the transition zone between light limited and light saturated CO_2 uptake (Fig. 14).

In contrast to winter plants, spring plants showed a pronounced daily pattern in both, CO_2 uptake and transpiration (Fig. 17). A marked depression in the rate of CO_2 uptake was observed after 7-8 h light. The depression was not so strong in the youngest leaves, but there it may have been masked by their high growth rate. Possible causes of this depression will be discussed in Section 5.6.

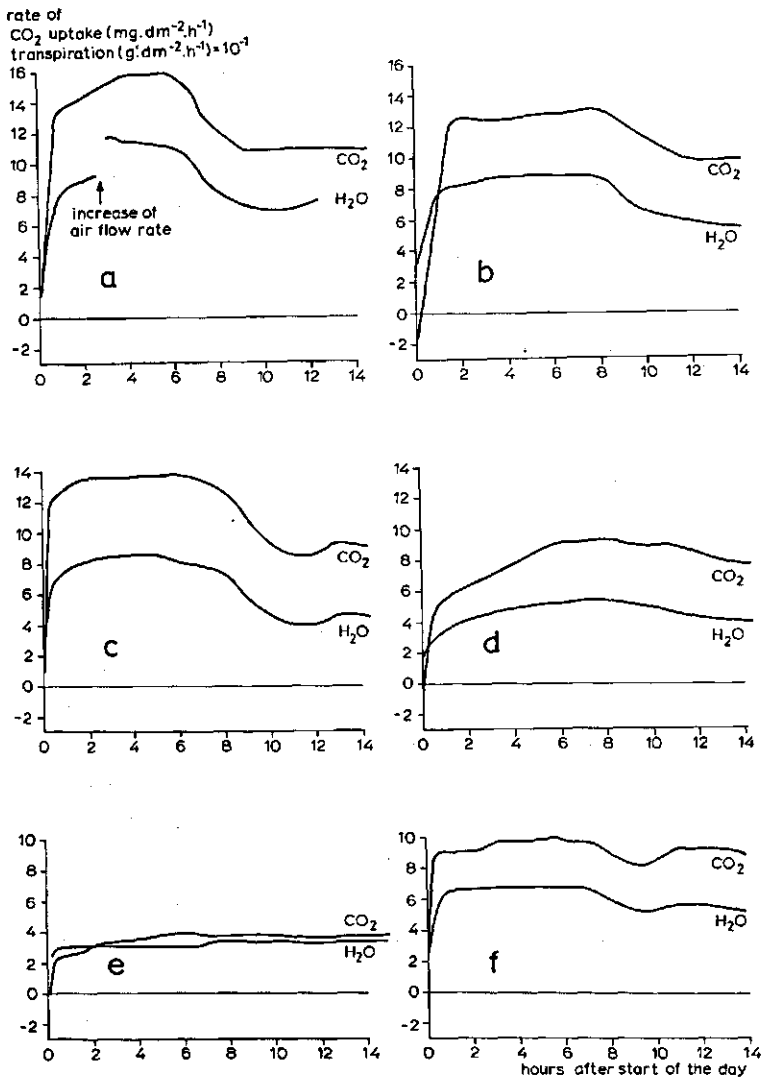

Fig. 17. Daily course of the rate of CO_2 uptake and of transpiration of different leaves and of a complete shoot of 5-leaf spring plants under standard conditions. a. leaf 1, b. leaf 2, c. leaf 3, d. leaf 4, e. leaf 5, f. complete shoot.

Daily total amount of CO_2 uptake by different leaves of spring plants are presented in Table 13. Rate of CO_2 production during the day was estimated by averaging initial and end values. Rate of CO_2 uptake and estimated rate of CO_2 production were added to calculate 'gross photosynthesis'. Total amount of CO_2 taken up by standard plant leaves was calculated according to the procedure described in the introduction of this section. These values are given in Table 13.

Table 13. Rate of CO_2 exchange, calculated rate of 'gross photosynthesis' of different leaves of 5-leaf spring plants, measured at 25 °C, 300 $\mu l.l^{-1}$ CO_2 concentration and 75 $W.m^{-2}$ irradiance, and the daily amount of CO_2 uptake by standard plant leaves calculated from the first column.

Leaf No.	a CO_2 uptake ($mg.dm^{-2}.14\ h^{-1}$)	b CO_2 production ($mg.dm^{-2}.14\ h^{-1}$)	a+b 'Gross photosynthesis' ($mg.dm^{-2}.14\ h^{-1}$)	CO_2 uptake of standard plant leaves ($mg.leaf^{-1}.14\ h^{-1}$)
cotyledons	.	.	.	59[1]
1	181	25	206	328
2	161	29	191	631
3	162	24	187	675
4	112	31	143	300
5	48	56	104	38
6	.	.	.	8[1]

1. These values were estimated from the closest leaf.

CO_2 uptake of all leaves of the standard plant together was thus estimated at 2037 mg $CO_2.14\ h^{-1}.plant^{-1}$. Stem respiration was not measured but on the basis of growth rate and chemical composition it was estimated according to the method described in Section 5.3. Subtracting the resulting 49 mg $CO_2.stem^{-1}.14\ h^{-1}$ from the total CO_2 uptake of the leaf that of the whole shoot was estimated at 1988 mg $CO_2.shoot^{-1}.14\ h^{-1}$. In an experiment with a whole shoot in which, for technical reasons an irradiance of 65 $W.m^{-2}$ was used, an amount CO_2 uptake was measured which yielded a standard plant value of 1975 mg $CO_2.plant^{-1}.14\ h^{-1}$, which compares well with the estimate obtained from single leaf measurements.

4.2 PRODUCTION OF CO_2 BY DIFFERENT ORGANS AND BY COMPLETE SHOOTS

CO_2 production in course of time was measured on individual leaves, the complete shoot and on the root system, including the hypocotyle. During these experiments, the organs remained attached to the plant, which was always under controlled environmental conditions. These precautions were taken, because it was expected that changes in other organs might influence CO_2-producing processes in the organ investigated.

CO_2 production by photosynthesizing organs was only measured during the night. Dark respiration processes probably continue in the light (Mangat et al., 1974) but because at the same time photosynthetic processes are producing large amounts of energy equivalents, their separate measurement is not only

very difficult but also less important in understanding the energy relations in the plant.

The results were converted to standard plant values on a dry weight basis, allowing for the weight increase of plants during the measurement. This procedure, however, was not followed with the individual leaf measurements, because here a continuous shift, related to plant size may be expected in the relative contribution of different processes to leaf respiration.

4.2.1 Diurnal course of the rate of CO_2 production in winter plants

Time course of CO_2 production of the root during a day/night cycle and of a complete shoot and of its separate organs during the night, are presented in Fig. 18. A common feature in all organs is the strong depression of the rate of CO_2 production after 10 or 11 h darkness, which was correlated with substrate depletion (Chapter 5, Section 4.3). This phenomenon could also be observed in spring plants, when the night was prolonged by some hours. The leaves were characterized by a high initial respiration rate, which gradually declined. There is a great similarity in the patterns of leaves 1 and 2 and the root system.

Initiation of the day is reflected in CO_2 production of the root by a sudden drop in its rate and similarly start of the night by a small increase,

Table 14. CO_2 production of organs of winter plants under standard conditions.

Organ	CO_2 production during the night		Average rate
	($mg.organ^{-1}.night^{-1}$)	($mg.dm^{-2}.night^{-1}$)	($mg.dm^{-2}.h^{-1}$)
leaf 1	5.70	8.92	0.558
leaf 2	9.42	7.40	0.462
leaf 3	9.71	9.24	0.578
leaf 4	10.15	13.70	0.856
leaf 5	2.29	21.80	1.363
complete shoot	68.90	12.68	0.793
	CO_2 production during day and night		
	($mg.organ^{-1}$. time $interval^{-1}$)	($mg.g^{-1}.h^{-1}$)	($mg.g^{-1}.h^{-1}$.
roots			
night	27.0	176.3	11.0
daytime	14.4	93.8	11.7
24 h	41.4	270.1	11.3

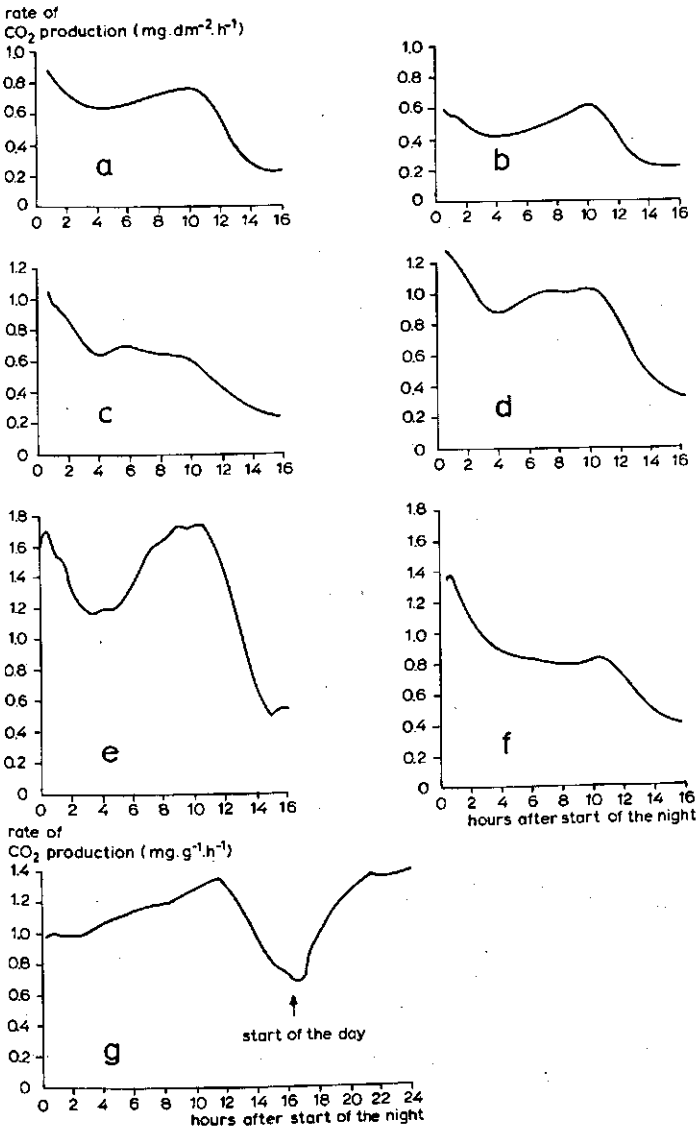

Fig. 18. Diurnal course of CO_2 production of organs of winter plants under their standard conditions. a. leaf 1, b. leaf 2, c. leaf 3, d. leaf 4, e. leaf 5, f. complete shoot, g. root system.

phenomena which probably are related to the water status of the plant. Winter plants needed a long time to recover from the depression in CO_2 production of the root, which occurred at the end of the night.

In Table 14 CO_2 production of different organs has been integrated over day and night. On the basis of these data, CO_2 production of the complete shoot and the root system of standard winter plants was calculated. Thus a

value of 54.3 mg CO_2 was obtained for the complete shoot (night) and 23.5 (night) and 12.5 (daytime) for the root system.

4.2.2 *Diurnal course of the rate of CO_2 production in spring plants*

Time course of the rate of CO_2 production of the root during a day/night cycle and of a complete shoot and of its organs during the night, are presented in Fig. 19. Like winter plants, spring plants show an initial phase in

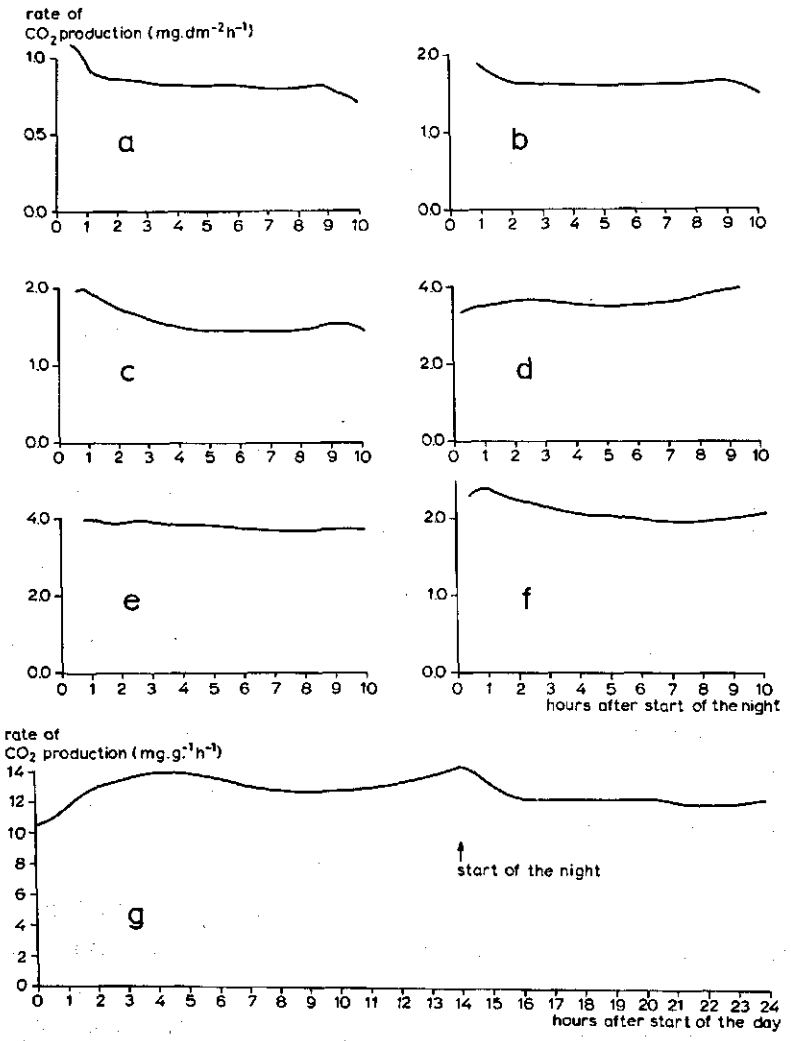

Fig. 19. Diurnal course of the rate of CO_2 production of organs of spring plants under their standard conditions. a. leaf 1, b. leaf 2, c. leaf 3, d. leaf 4, e. leaf 5, f. complete shoot, g. root system.

Table 15. CO_2 production of organs of spring plants under standard conditions

Organ	CO_2 production during the night		Average rate
	($mg.organ^{-1}.night^{-1}$)	($mg.dm^{-2}.night^{-1}$)	($mg.dm^{-2}.h^{-1}$)
leaf 1	11.7	8.42	0.842
leaf 2	30.8	16.4	1.64
leaf 3	30.5	15.9	1.59
leaf 4	28.2	36.2	3.62
leaf 5	16.9	38.2	3.82
complete shoot	185	20.3	2.03
	CO_2 production during day and night		
	($mg.organ^{-1}$.time interval^{-1})	($mg.g^{-1}.h^{-1}$)	($mg.g^{-1}.h^{-1}$
roots			
night	85.6	123.5	12.4
daytime	128.2	185.0	13.2
24 h	213.8	308.5	12.9

most leaves, in which CO_2 production decreases gradually. But this phase is much less pronounced. No further changes are observed, until a small rise in CO_2 production occurs which, as in winter plants, precedes a sharp decline. This decline, however is interrupted in spring plants, at the start of the day. CO_2 production of the root readily responds to the light but is otherwise essentially constant.

In Table 15 the amount of CO_2 production of different organs, integrated over day and night is presented. The values for leaves pressed in mg CO_2. $dm^{-2}.h^{-1}$ are much higher than the comparable data for winter plants. In contrast the values for the root system are quite close to those of the winter plants. CO_2 production in the standard plant was calculated according to the procedure described in Section 4.2, on a dry weight basis. Thus a value of 234 mg CO_2 per night was found for the shoot and 155 mg CO_2 (daytime) and 104 mg CO_2 (night) for the roots.

4.3 STARCH AND SUGAR RESERVES IN LEAVES, STEM AND THE ROOTS

Starch and sugar content in the leaves, the stem, including the hypocotyle and in the roots of plants growing under their standard conditions in the phytotron, were determined according to the methods described in Section 2.3.1. Two-plant samples were taken every 2 h of a day/night cycle (every

4 h for the night of the winter plant. Levels in different plants were so similar that statistical error must have been small in comparison to the changes observed between different harvesting times.

The results are presented in Fig. 20. Most remarkable is the very reg-

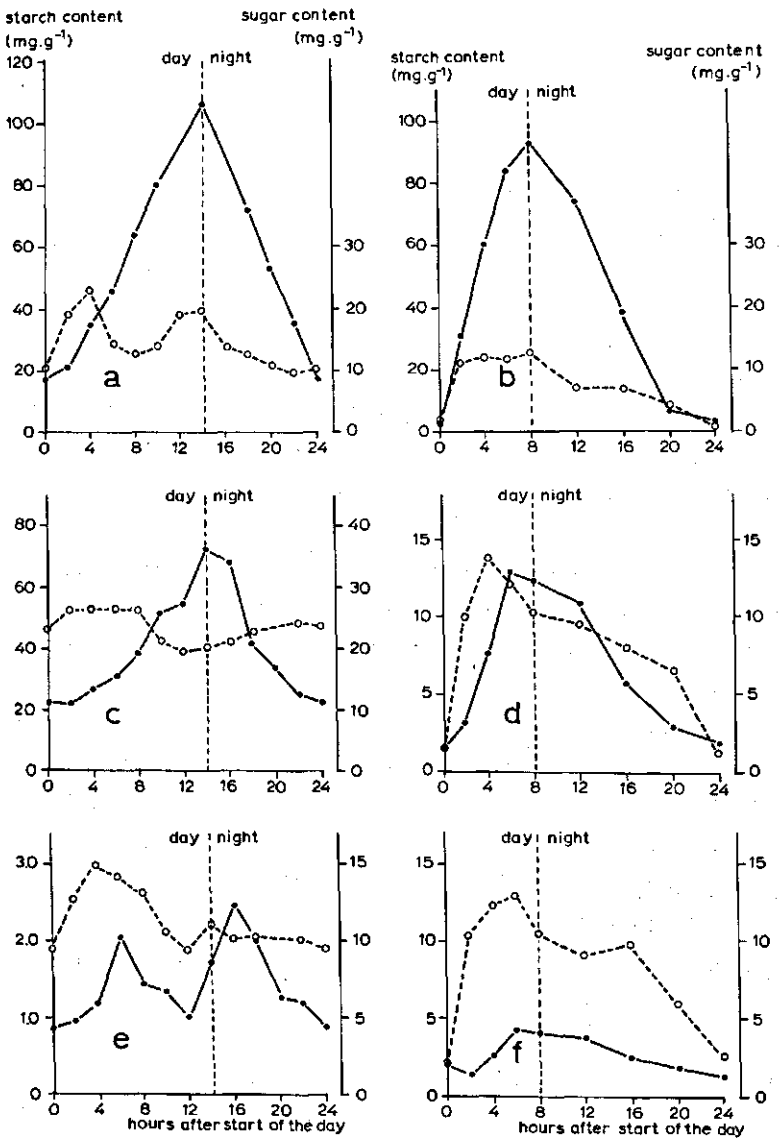

Fig. 20. Diurnal course of starch (•—•) and sugar contents (o--o) in the leaves, (a, b), the stem (including petioles, hypocotyle and growing point) (c, d) and the roots (e, f) of 5-leaf spring (a, c, e) and winter plants (b, d, f) growing under their standard conditions. Contents are expressed on basis of starch and sugar free dry weight.

ular course of starch content in the leaves, which is also weakly reflected in the stem. In leaves of winter plants the constant rate of starch breakdown in the night resulted in a premature depletion of these reserves. This in turn, led to a reduction of sugar content in all plant parts. Starch content in the root was comparatively low, but its sugar content differed little from that in other plant parts.

In contrast to starch, sugar content showed a less well defined pattern. In leaves of winter plants, sugar content was much higher during the day than during the night, but in those of spring plants in the daytime a very pronounced depression was observed after 6 h, which cannot easily be connected with other phenomena.

Rate of formation or consumption of starch and sugar in standard plants during the interval between two harvests can be calculated, provided dry weight of different plant parts at the moment of harvesting are known. Dry

Table 16. Amount of starch and sugars at different times of day and night, in different parts of the standard plant expressed in mg per plant part; time is expressed in hours after start of the day.

Time	Leaves		Stem + hypocotyle		Roots		Total
	starch	sugars	starch	sugars	starch	sugars	reserves
Winter plant							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	14.53	4.69	0.31	1.53	-0.04	0.48	21.50
4	30.07	5.30	1.14	2.28	0.04	0.62	39.45
6	43.73	5.31	2.22	2.07	0.14	0.68	54.15
8	50.07	6.09	2.22	1.81	0.14	0.54	60.87
12	39.62	2.89	1.93	1.66	0.12	0.45	46.67
16	20.00	2.92	0.85	1.34	0.03	0.50	25.64
20	2.43	1.36	0.28	1.04	-0.01	0.24	5.34
24	0.72	-0.43	0.08	-0.04	-0.05	0.02	0.30
Spring plant							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	8.73	18.82	-0.03	1.5	0.05	1.62	30.69
4	38.61	28.39	0.24	1.66	0.17	2.84	71.91
6	67.37	9.25	0.48	1.81	0.65	2.56	82.12
8	112.61	5.78	0.98	1.79	0.33	2.12	123.61
10	159.08	9.68	1.84	-1.29	0.28	0.67	170.26
12	.	.	2.16	-2.53	0.08	-0.02	.
14	243.60	26.06	3.47	-2.03	0.53	1.12	272.75
16	.	.	3.19	-1.41	1.05	0.44	.
18	149.73	6.80	1.31	-0.27	0.74	0.55	158.86
20	96.90	1.85	0.79	0.25	0.26	0.44	100.49
22	49.22	-1.67	0.19	0.65	0.22	0.43	49.04
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00

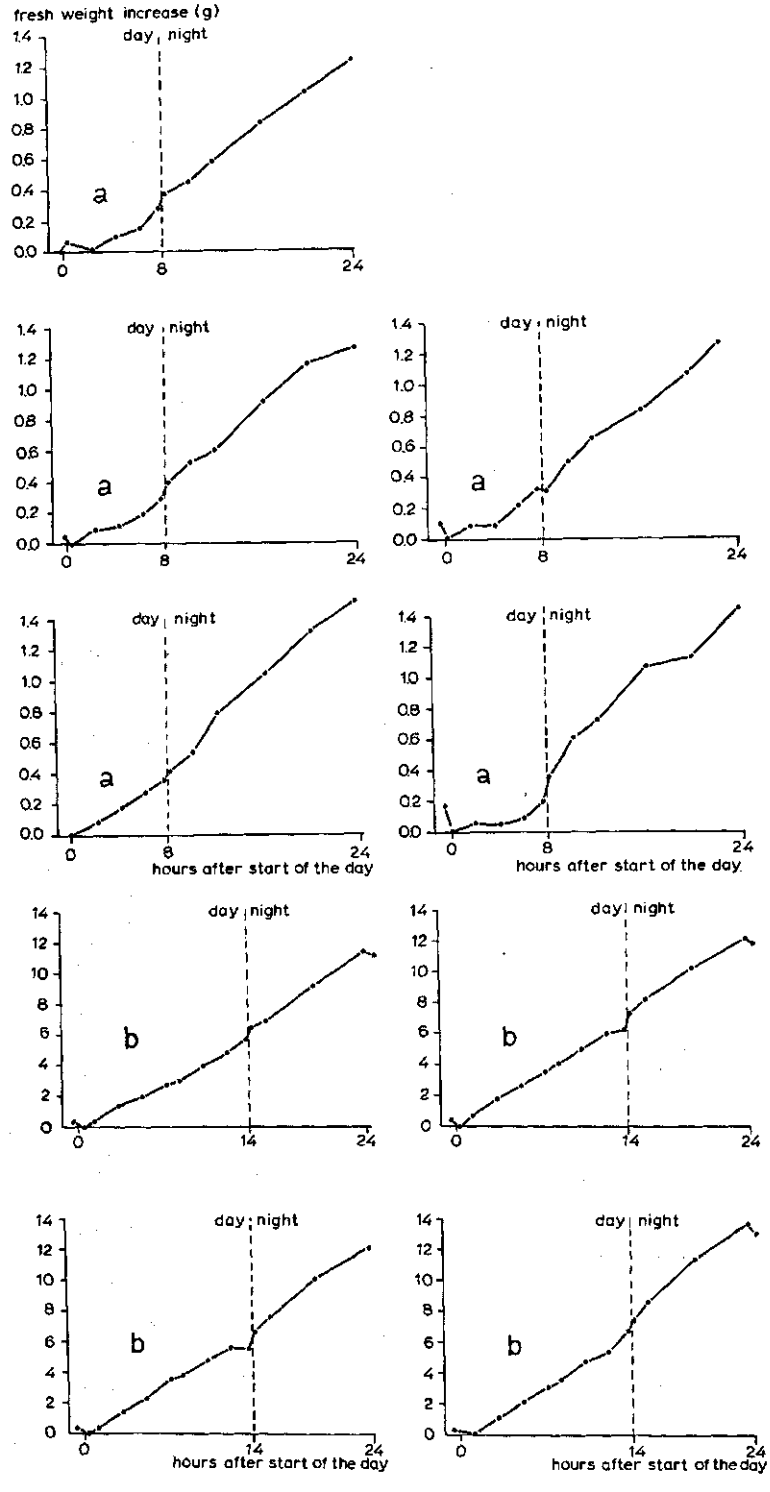

Fig. 21. Fresh weight increase of 5-leaf winter (a) and spring plants (b) during a day/night cycle. Each curve represents one experiment.

weight of these parts will change, as a result of growth and reserve accumulation. Because the time course of growth of different plant parts was not known, dry weight had to be estimated. For these calculations dry weight was assumed to increase during the day only, at a constant rate (Table 16).

4.4 GROWTH IN FRESH WEIGHT

Fresh weight of winter and spring plants was measured by periodically weighing plants growing under standard conditions, using the procedure described in Section 2.4. Fig. 21 shows fresh weight increase of winter and spring plants as a function of time. As weight increase was small in comparison to plant weight, water status influenced the results considerably. For this reason fresh weight measurements were made 15 min before and after the transients between day and night to allow for changes in water content.

Although there was much scatter in the data, it may be concluded that rate of fresh weight increase was higher during the night than during the day. This result agrees with the observations of Kleinendorst & Brouwer (1970), for example, on leaf growth of maize. The growth rate was highest in winter plants in the hours before and after the onset of the night. In spring plants maximum growth rate was observed in the first hours of the night.

Total increase in fresh weight over 24 h established by this method yielded much lower values than the periodical harvest method: the weight increase was 11% compared with 17% for winter plants, and 26% instead of 40% for spring plants. Therefore patterns described here may not be representative for the situation in undisturbed plants!

5 Interpretation and discussion

Plant growth may be represented by the scheme in Fig. 22. In the light, plants take up CO_2 and use it for the formation of primary building blocks, such as sugars and amino acids (Process a). Part of these compounds may be stored (Process b) and another part is transformed into more complex compounds, such as cellulose and proteins, forming thus the structural dry weight of the plant (Process c). Within narrow limits, the increase of fresh weight (Process d) will be ultimately dictated by the increase in structural dry weight. In darkness, the direction of Process b is reversed and stored photosynthetic products will be available for the formation of structural dry weight and to meet the energy requirements of the plant.

In this scheme, the increase of structural dry weight is central whereas the relation between structural dry weight and fresh weight is considered as a long-term adaptation to the overall environmental conditions, along with other morphological plant characteristics.

Under constant environmental conditions, without alternating day and night a stationary situation might occur, in which the measurement of net CO_2 uptake would be sufficient for the determination of growth rate if the carbon content of the plant is known. Normally plants are growing under alternating day and night periods and thus the situation is not stationary: the size of different pools depicted in the scheme in Fig. 22 may vary considerably, and the rate of Process c should be measured rather than Process a.

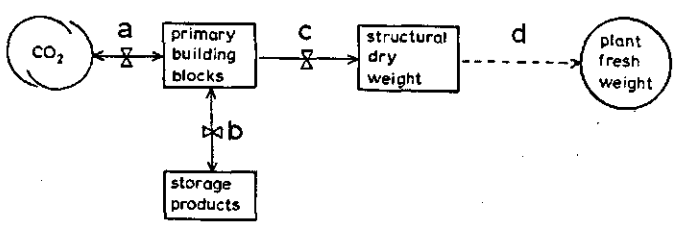

Fig. 22. Schematic representation of plant growth processes in relation to CO_2 uptake. a = CO_2 exchange, b = rate of storage product formation/break down, c = rate of structural dry weight formation, d = relation between structural dry weight and plant fresh weight.

Theoretically the diurnal course of Process c could be measured by harvesting plants successively and determining the amount of different plant constituents at these moments. Very many plants would be required as well as high precision analysis to obtain any reasonable result.

An indirect approach to this problem is to calculate the amount of carbon incorporated in Process c, by comparing CO_2 exchange and changes in the size of the storage pool and the primary building-block pool. This C-balance method requires the measurement of the size of these pools, mainly represented by starch and sugars. It can only be used for whole plants. For individual organs the rate of carbon exchange with other plant parts must be known. For short-term growth studies a more convenient way of measuring growth rate of plants or plant parts could be of great value.

In this chapter the possibilities to adopt the rate of CO_2 production as an indicator of the rate of structural dry weight formation will be investigated and the results will be compared with those obtained with the C-balance method. The substrates used and the major products formed during the growth process will be discussed in the first section. Later in this chapter attention will be paid to the interpretation of the diurnal course of CO_2 exchange.

5.1 SUBSTRATE FOR GROWTH AND RESPIRATION, AND CHEMICAL COMPOSITION OF THE DRY WEIGHT ACCRETION

The green parts of plants trap light energy and convert it into chemical energy. This chemical energy is used for the reduction of CO_2, NO_3^- and SO_4^{2-} and subsequent formation of sugars and amino acids. Furthermore it is utilized in the formation of starch and transport of sugars and amino acids, whereas in growing leaves it directly contributes to the energy requirements of growth processes (Penning de Vries, 1975a).

Chemical energy is also provided by the respiration processes, in which sugars formed during photosynthesis are consumed. Unavoidably less energy is produced when a certain amount of sugars is broken down than is required for its formation. Penning de Vries (1975a) indicated, that whereas the formation of 1 mol of glucose requires 54 mol ATP (adenosine triphosphate) equivalents, its breakdown is associated with the formation of only 38 mol ATP. Thus 30% energy is lost when sugar acts as an intermediate compared with when photosynthetic energy is directly used.

Unfortunately energy provided by the photosynthetic process, which is in the

form of ATP and $NADPH_2$ (reduced form of nicotinamide adenine dinucleotide phosphate) can neither be stored nor transported to other plant parts as such. Thus heterotrophic plant parts, and in darkness the whole plant, are dependent for their energy supply on sugar and starch formed in the leaves in the light.

A process requiring much energy in plant growth is the reduction of NO_3^-. Therefore, in view of the remarks made above, it would be profitable for the plant to use photosynthetic energy directly for this process. Indeed, Beevers & Hageman (1969) provided much evidence that although plants, when supplied with sufficient sugars, are able to reduce NO_3^- in darkness (Berner, 1971), this process occurs mainly in the light, in the leaves of many cultivated plants. My observations supported the idea that in winter plants NO_3^- reduction also takes place mainly during the day. When 5-leaf winter plants were kept during the night on a nutrient solution in which NO_3^- had been replaced for 50% by SO_4^{2-} and for 50% by Cl^-, although in most of their organs abundant NO_3^- was available, no NO_3^- consumption could be detected over a period of 16 hours (Table 17).

In the standard plant 0.40 mg NO_3^- must be reduced per 24 h for protein growth (Chapter 3) in comparison with which the amount reduced during the night, if any, is very small. The assumption that NO_3^- is reduced exclusively in the leaves in the light has some important consequences, when the chemical composition of structural dry weight accretion is considered in the course of time.

Dijkshoorn (1973) provided much evidence for the idea that in the processes associated with NO_3^- reduction as may organic acid equivalents are generated as NO_3^- equivalents are reduced, and that when nitrogen is supplied as NO_3^-, these processes are the main source of organic acid formation. Organic acids thus are formed in the leaves in the light.

If NO_3^- is reduced only in the light, the amount of protein can increase in darkness only at the expense of stored amino acids (NH_3 normally does not

Table 17. NO_3^- content in winter plants, before and after a night on a NO_3^--free nutrient solution.

Time	Number of plants	Plant dry weight ($mg.plant^{-1}$)	NO_3^- content ($mequiv.g^{-1}$ dry weight)	NO_3^- amount ($mequiv.plant^{-1}$)
start of the night	14	787	1.068	0.84
end of the night	14	776	1.065	0.83

accumulate in plants). Although some fluctuations in the content of free amino acids have been observed by various authors, the amounts involved are negligible in comparison with the total increase of protein over 24 h (Durzan, 1968a,b; Noguchi & Tamaki, 1962).

Therefore protein formation during the night is possible only at the expense of other proteins. Indeed various authors have observed a decrease in the amount of proteins in the leaves during the night (McKee, 1958). Because proteins as such are not transported in plants, redistribution must be associated with breakdown and resynthesis, amino acids and amides being the intermediates which are transported.

As has been stated before, during NO_3^- reduction, as many equivalents organic acids are formed as equivalents NO_3^- are reduced. In some plants like sugar beet, potato and tomato plants (Dijkshoorn, 1973), all organic acids formed in this way stay in the plant, whereas Van Egmond (1975) demonstrated for sugar beet, that they accumulate in the leaves where they are synthesized. In other plants these acids are partly transported to the roots, where they are decarboxylated, whereas the remaining carbon skeletons are further metabolized (Dijkshoorn, 1973; Penning de Vries et al., 1974).

The fraction of organic acids decarboxylated in the roots can be estimated by comparison of the amount of organic acids and organic nitrogen in the plant. As these substances are formed in equivalent amounts and plants normally do not lose appreciable amounts of organic nitrogen, the difference between the contents of organic acid and organic nitrogen may be explained by decarboxylation of the former (Table 18, p. 54).

Table 18 shows that in winter plants at the end of the night a somewhat higher fraction of organic acids is decarboxylated than at the end of the day, suggesting that some decarboxylation occurs during the night. In the further calculations, rather arbitrarily it will be assumed that all decarboxylation occurs during the daytime, but the effect of this assumption is small.

Probably an important fraction of root respiration is caused by microorganisms, living on the roots, as will be shown later in this chapter. Substrate for these organisms may not only be provided by leakage of organic compounds from the roots but also by dying root cells (Rovira, 1969). The contribution of either group of substrates is not known.

Under conditions of prolonged darkness, reserve carbohydrates will be depleted and then starvation gradually sets in. Under these conditions protein breakdown provides substrate for respiration, whereas organic nitrogen

Table 18. Decarboxylation of organic acids. Data are expressed in mequiv.g^{-1} plant dry weight.

	1	2	3	4	5	6	
winter plants (samples of 10 plants)							
end of light period	3.62	1.14	2.48	4.13	2.99	0.51	(17%)
end of dark period	3.95	1.38	2.57	4.56	3.18	0.61	(19%)
spring plants (derived from Chapter 3)							
end of dark period	11.4	3.01	8.40	-	10.7	2.3	(21%)

1. Ash alkalinity.
2. NO_3^- content.
3. 1 - 2 = C-A or organic acid content.
4. Total N-content.
5. 4 - 2 = organic N-content.
6. 5 - 3 = shortage of organic acids, ascribed to decarboxylation, also expressed as a percentage of 5, or the amount of organic acids formed.

accumulates as amides (Yemm, 1965). In winter plants, after 12 h of darkness, carbohydrate reserves were depleted and Table 19 shows, that during the last 4 h of the night the amount of carbon lost by respiration exceeded the amount of carbon consumed from the carbohydrate reserves. Breakdown of substances classified as structural must be responsible for this discrepancy, and in view of the literature cited above, it may be assumed that these substances are proteins.

A summary of this discussion gives the following picture: Energy and substrate are required for growth. Energy is directly obtained from photosynthesis in the green parts in the light, from respiration in heterotrophic parts, and also from respiration in autotrophic parts in darkness. Substrates for respiration are sugars and under starvation conditions also amino acids. In the roots organic acids after decarboxylation may act as a respiration substrate.

Substrates for growth in the light in autotrophic parts are CO_2 and NO_3^- and in darkness and in heterotrophic parts sugars and amino acids. In the light amino acids and sugars are stored as proteins and starch, whereas in darkness the direction of these processes is reversed. In Fig. 23 most of these processes are visualized.

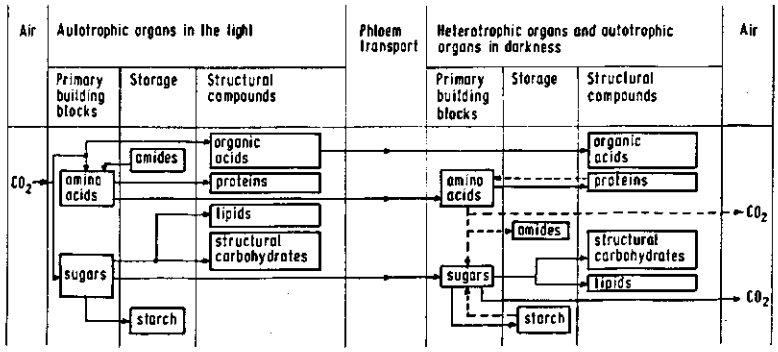

Fig. 23. Schematic representation of carbon flow through different plant compounds during day and night. ----▸ = processes which take place in darkness only.

5.2 CARBON INCORPORATION IN STRUCTURAL DRY WEIGHT IN STANDARD SPRING AND WINTER PLANTS

In Chapter 4 the diurnal course of CO_2 exchange and of the amount of carbohydrate reserves in standard spring and winter plants has been described. Because these data all refer to standard plants, they may be compared. This enables us to calculate the rate of carbon incorporation in structural dry weight, according to:

$$I = P - R - F,$$

where I = rate of carbon incorporation in structural dry weight
P = rate of CO_2 uptake
R = rate of CO_2 production
F = rate of formation of reserve carbohydrates

These rates are expressed as mg C per time interval. In this approach the comparison of these results obtained by different techniques involves some risks due to systematic errors. With a time interval of 24 h the results of this comparison can be checked, because the calculated amount of carbon incorporation in the structural dry weight can be derived from the data on growth rate and chemical composition given in Chapter 3, together with the data of Table 19.

Thus C content in the structural dry weight accretion was 0.356 $g.g^{-1}$ in winter plants, and 0.376 $g.g^{-1}$ in spring plants. Total C-consumption by standard spring and winter plants in 24 h was calculated, the results being given

Table 19. C content of different plant constituents, with chemical composition of each fraction according to Penning de Vries et al. (1974).

Fraction	C content ($g.g^{-1}$)
organic N-compounds	0.544
lipids	0.772
carbohydrates	0.450
lignin	0.639
organic acids	0.364
minerals	0.000

in Table 20.

The amount of carbon used for growth and CO_2 production in 24 h was 68.6 mg C or 252 mg CO_2 for winter plants compared with 233 mg CO_2 calculated from gas exchange measurements. Likewise, spring plants require 579 mg C or 2123 mg CO_2 to meet their demands as compared with 2037 mg CO_2 calculated from gas exchange measurements. The values obtained from the carbon balance thus compare well with those from the gas exchange experiments and will be further used.

In the previous section evidence was given in favour of the assumption that all organic nitrogen compounds and organic acids, in the plants studied here, are formed during the daytime. This together with the knowledge about carbohydrate reserve formation during the day makes it possible to calcu- and lipids. In this way the distribution of structural carbohydrate formation over day and night can be estimated (Table 21).

In this way the distribution of structural carbohydrate formation over day and night can be estimated (Table 21).

In the carbon balance of winter plants the breakdown of proteins at the

Table 20. C-consumption of standard spring and winter plants per 24 h.

winter plants:	growth	42.3 mg C
	root respiration (day and night)	9.8 mg C
	shoot respiration (night)	16.5 mg C +
	total C-consumption	68.6 mg C
spring plants:	growth	445 mg C
	root respiration (day and night)	71 mg C
	shoot respiration (night)	63 mg C +
	total C-consumption	579 mg C

Table 21. Carbon balance of standard spring and winter plants, during the day and the night.

	Consumption (mg C.time interval^{-1})		Supply (mg C.time interval^{-1})
Winter plants			
Day			
CO_2 uptake			68.6
starch and sugar formation	29.6		
root respiration	3.4		
organic acid growth	6.2		
organic nitrogen compound growth	18.8		
total known C-consumption	58.0	+ ⟶	58.0 −
C-incorporation in structural carbohydrates, lignins and lipids			10.6 (56%)[1]
Night			
starch and sugar consumption			29.6
protein breakdown			1.5 +
root respiration	6.4		31.1
shoot respiration	16.5		
total known C-consumption	22.9	+ ⟶	22.9 −
C-incorporation in structural carbohydrates, lignins and lipids			8.2 (44%)[1]
Spring plants			
Day			
CO_2 uptake			579
starch and sugar formation	133		
root respiration	42		
organic acid growth	68		
organic N-compound growth	184		
total known C-consumption	427	+ ⟶	427 −
C-incorporation in structural carbohydrates, lignins and lipids			152 (78%)[1]
Night			133
starch and sugar consumption			
root respiration	28		
shoot respiration	63		
total known C-consumption	91	+ ⟶	91 −
C-incorporation in structural carbohydrates lignins and lipids			42 (22%)[1]

1. The percentages indicate the relative distribution of carbon incorporation in structural carbohydrates, lignins and lipids over day and night.

Table 22. Carbon balance of standard spring and winter plants in 2 or 4 h intervals in the course of day and night. All data are expressed in mg C per time interval.

Time interval (h after start of the day)		a CO_2 uptake	b CO_2 production by the root	c starch and sugar formation	a-b-c C incorporation in structural dry weight
winter plant					
0 - 2		17	0.58	10.4	6.2
2 - 4	daytime	17	0.84	8.8	7.6
4 - 6		17	0.99	7.3	8.9
6 - 8		17	0.99	3.3	12.9
		d starch and sugar consumption	e CO_2 production by the root	f CO_2 production by the shoot	d-e-f C incorporation in structural dry weight
8 - 12		7.0	1.5	5.7	-0.2
12 - 16	night	10.3	1.6	4.2	4.5
16 - 20		9.9	1.9	4.0	4.0
20 - 24		2.5	1.4	2.6	-1.5
		a CO_2 uptake	b CO_2 production by the root	c starch and sugar formation	a-b-c C incorporation in structural dry weight
spring plant					
0 - 2		90	5.3	15	70
2 - 4		90	6.2	20	64
4 - 6		90	6.4	5	79
6 - 8	daytime	90	6.1	21	63
8 - 10		84	5.9	23	55
10 - 12		70	6.0	24	40
12 - 14		63	6.2	26	31
		d starch and sugar consumption	e CO_2 production by the root	d CO_2 production by the shoot	d-e-f C incorporation in structural dry weight
14 - 16		31	6.2	14	11
16 - 18		25	5.7	13	6
18 - 20	night	28	5.7	12	10
20 - 22		25	5.4	12	8
22 - 24		24	5.4	12	7

end of the night was taken into account. This resulted in an extra carbon supply of 1.5 mg C (Table 22) during the night and extra carbon incorporation in organic nitrogen formation during the daytime, where accumulated amides

are converted back into proteins (Fig. 23).

Table 21 shows that even though the day was much shorter, winter plants used 43% of their CO_2 uptake for the formation of carbohydrate reserves, compared with only 23% in spring plants. Synthesis of structural carbohydrates, lignins and lipids continued during the night, but its rate of formation was much less than in the daytime. It shows, moreover, that stored carbohydrates during the night were used mainly for respiration: 72% in winter plants and 68% in spring plants was lost by respiration.

Carbon taken up during the day was converted into structural dry weight with an efficiency of 62% in winter plants and 77% in spring plants. These values compare well with those given by McCree (1974), Penning de Vries (1975a) and Ryle et al. (1976).

The approach used in Table 21 also can be applied over shorter time intervals. However, because the time course of NO_3^- reduction and its associated processes is not known, the different growth processes cannot be distinguished during the daytime.

Table 22 reveals a somewhat different behaviour of spring and winter plants in their distribution of assimilated carbon over storage and structural products. In winter plants in the course of the day the balance between storage and structural products was gradually shifted towards the latter. In spring plants, in spite of a decreasing rate of CO_2 assimilation, an increasing amount of storage products was formed at the expense of the formation of structural products.

During the night also some differences between spring and winter plants could be observed. Whereas the rate of carbon incorporation in structural dry weight was essentially constant during the night in spring plants, strong fluctuations did occur in winter plants. The negative value found in these plants in the last four hours of the night may be ascribed to depletion of reserves and associated starvation processes, as has been explained in the previous section. For the situation observed in the first four hours of the night, however, no explanation can be given.

It should be stressed at this point that although basically sound, the approach followed had a serious drawback: in the calculation of the rate of carbon incorporation in structural dry weight, relatively small errors in the basic data would be strongly magnified in the final results. In spite of this, the general tendencies indicated by this analysis seem to be well established.

5.3 CO_2 PRODUCTION OF STANDARD SPRING AND WINTER PLANTS DURING THE NIGHT

When plants are entering the dark period, their direct energy and substrate supply via photosynthesis is cut off. Biosynthetic processes, however, continue at the expense of stored carbohydrates. The role of these carbohydrates is twofold: they are used as building blocks and as an energy source. It is possible to calculate the amount of glucose, required as building blocks for the formation of a certain amount of plant compound if the pathways of biosynthesis are known. Knowledge of these pathways also enables one to calculate the associated amount of CO_2 production or consumption, due to decarboxylation or carboxylation reactions in the re-formation of carbon skeletons. Furthermore, the total amount of energy required for the formation of a certain amount of compound may be derived from it. The amount of energy generated in the complete oxidation of 1 mol glucose, as well as the associated amount of CO_2 production are well established. Therefore it is possible to calculate the amount of glucose required and the total amount of CO_2 evolved in the production of a certain amount of compound, for all products of which the biochemical pathways of formation are known (Fig. 24). Penning de Vries (1975a) carefully analysed this problem, and found that for reasonable calculations of CO_2 production associated with the formation of a certain amount of organic dry matter, it is sufficient to distinguish 5 major groups of compounds: organic N-compounds, lignins, lipids and organic acids. The exact

Table 23. CO_2 production associated with the formation of various plant compounds in darkness, calculated according to Penning de Vries and his co-workers.

Compound formed	CO_2 production ($g.g^{-1}$)	Substrate
organic N-compounds	0.844	from a mixture of amino acids derived from zein[1]
organic N-compounds	1.78	NO_3^- and sugars[2]
organic N-compounds	0.591	proteins, amides being the intermediates[3]
carbohydrates	0.175	sugars[1]
lignin	0.620	sugars[1]
lipids	1.618	sugars[1]
organic acids	-0.0453	sugars and CO_2[1]

1. Penning de Vries (1975a)
2. Penning de Vries et al. (1974)
3. Penning de Vries & Van Laar (1976).

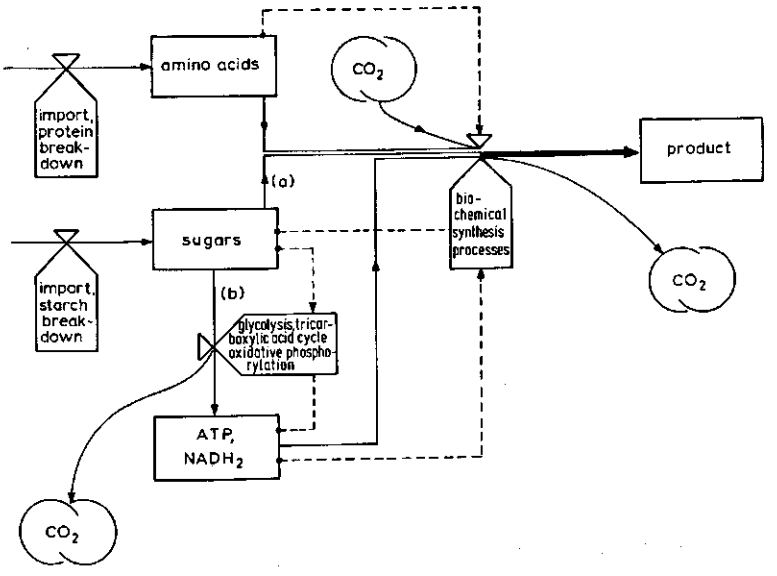

Fig. 24. Schematic representation of the relation between CO_2 production and formation of plant compounds in an organ. It shows the dual role played by sugars in supplying substrate for growth (a) and for energy production (b). The rectangles represent amounts and the valve symbols rates. Flow of matter is indicated by solid lines and information streams by broken lines.

chemical composition of each fraction proved to be of only minor importance for the results of these calculations. In Table 23 CO_2 production associated with the formation of 1 g of different compounds is given.

With the data presented here, one can calculate the amount of CO_2 production associated with the formation of a known amount of structural dry weight of a known chemical composition.

Vice versa, growth rate could be calculated from the amount of CO_2 production, if the chemical composition of the accretion is known and the contribution of other processes to CO_2 production have been quantified.

During the night, besides structural dry weight accretion, the following other processes may contribute to CO_2 production:

- transport processes
- maintenance processes
- idle respiration

Transport of organic molecules and minerals through membranes is generally an active process requiring energy. Sugars are transported mainly as sucrose, and the conversion of monomers into sucrose also requires energy. Taking these processes into account, Penning de Vries (1975a) came to the following minimum estimates of CO_2 production associated with transport pro-

Table 24. CO_2 production associated with active uptake or export of different substances, according to Penning de Vries (1975a).

Substances	CO_2 production per g substance transported ($g.g^{-1}$)
sugars (export, including sucrose formation)	0.0772
sugars (import)	0.0386
amino acids	0.0298
organic acids	0.0637
minerals	0.030

cesses (Table 24).

Maintenance processes are those processes that are not directly linked to growth, but which serve merely to maintain the structure of the organism. Maintenance of ionic and metabolic gradients in the tissue, and turnover of enzymes are their most important elements. At present, quantitative information about these processes and their regulation is poor (Huffaker & Peterson, 1974).

On the basis of literature data and theoretical considerations Penning de Vries (1975b) estimated that CO_2 production related to protein turnover is 41-78 mg $CO_2.g^{-1}$ protein.24 h^{-1}, or 11-21 mg $CO_2.g^{-1}$ dry weight.24 h^{-1}, and that CO_2 production related to maintenance of ionic gradients amounts to 9-15 mg $CO_2.g^{-1}$ dry weight.24 h^{-1}. Experimental data, however, showed a much wider range.

Idle respiration is respiration without any useful outcome. The occurrence and importance of such respiration is still the subject of discussion (Penning de Vries, 1974; Beevers, 1970).

Whereas the separation in various respiration components, as described above, theoretically seems to be satisfactory, some problems arise when applying it in practice. For example breakdown and resynthesis of proteins may be interpreted as growth but also as maintenance processes, and a distinction hardly seems possible. Furthermore with the approach used in this study it is not possible to distinguish between these processes, other maintenance processes and idle respiration.

To overcome these difficulties, Penning de Vries (1975a) made the following assumptions for the experimental test of this biochemical calculations:
- constant rate of maintenance respiration
- idle respiration, if any, occurring at a low and constant level
- constant chemical composition of structural dry weight accretion.

Table 22 demonstrates that in standard winter plants these conditions

are probably not met. In fact a high fraction of carbon incorporation in structural dry weight should be expected at high rates of CO_2 production, whereas the data of the first four hours of the night showed, that none, or only a negligible amount of carbon was incorporated. This discrepancy may be explained by changes in the rate of maintenance or idle respiration, or by shifts in the ratio between synthesis of proteins and that of the other components.

For a futher evaluation of the problem being considered, the size of different components of night respiration was calculated for the shoots of standard spring and winter plants. For the roots a separate calculation was carried out later.

In these calculations, because of the difficulties mentioned above, no distinction was made between protein metabolism, maintenance processes and idle respiration. Instead the known respiration components were calculated and subtracted from the measured rate of CO_2 production. The difference is discussed in relation to those less well determined processes (Table 25).

It was assumed that the ratio between structural carbohydrate, lipid and lignin formation was constant during day and night. With the carbon balances in Section 5.2 it could be deduced that the fraction of these compounds synthesized during the night, was 44% for winter plants and 22% for spring

Table 25. Contribution of different processes to CO_2 production of the shoot at night in standard spring and winter plants.

Process	Winter plants mg CO_2 plant^{-1}.16 h^{-1}		Spring plants mg CO_2 plant^{-1}.10 h^{-1}	
synthesis of:				
lipids	2.48		12.7	
carbohydrates	1.93		9.0	
lignins	0.76		3.5	
total	5.17	+	25.2	+
transport of:				
minerals	0.5		3.4	
sugars	5.2		23.4	
total	5.7	+	26.8	+
totally known	10.9	(18%)	52.0	(23%)
measured	60.5	(100%)	230	(100%)
difference	49.6	-	178	-
maintenance respiration	19	(31%)	64	(28%)
other processes	30	(50%)	114	(50%)

plants. Because of problems with methods, lignins were nog determined in the chemical analysis. Their content was estimated at 10% of the fraction carbohydrates.

Table 25 shows that during the night, in the shoot of standard spring and winter plants transport processes and synthesis of non-protein compounds each contributed only about 10% to the total amount of respiration. Thus the rate of respiration was determined essentially by protein metabolism, maintenance processes and possibly by idle respiration.

Excluding for a moment idle respiration as an important factor in determining the rate of CO_2 production, one can calculate the amount caused by the protein metabolism that is related to growth, if the maintenance processes can be evaluated.

For this purpose the relation between sugar contents in the leaves and rate of CO_2 production was considered (Fig. 25). In winter plants a linear relation was found, as described by Yemm (1965) and Penning de Vries et al. (1976). Both observed saturation at increased sugar contents and this in fact may have occurred in spring plants. Extrapolating the graph to the ordinate gives a positive rate of CO_2 production. If we assume that all growth and transport processes stop at this sugar content, this rate would be ascribed to maintenance respiration. It equalled 6 mg $CO_2.g^{-1}$ protein.h^{-1}, which was about twice the highest value given by Penning de Vries (1975b). It was also higher than the minimum respiration rate observed in the winter plant leaves, which expressed on a protein basis had a value close to 4.6 mg CO_2.

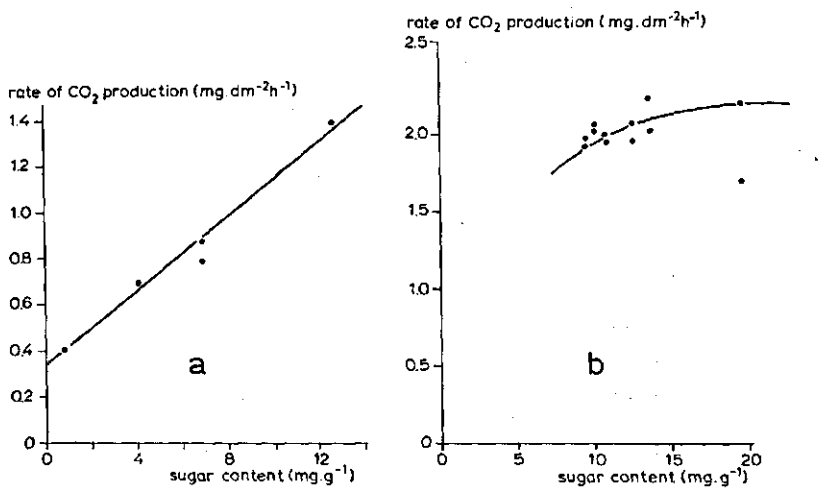

Fig. 25. Relation between sugar content in the leaves and rate of CO_2 production of the shoot. a. winter plants, b. spring plants.

g^{-1} protein.h^{-1} in all leaves.

Another method to estimate maintenance respiration is to extrapolate to the ordinate the relation between CO_2 production during the night and CO_2 uptake during the day (Fig. 26) (Penning de Vries, 1975b). In this way a value of 5 mg CO_2.g^{-1}protein.h^{-1} was obtained, which compares reasonably well with the previous value. The value of 6 mg CO_2.g^{-1} protein.h^{-1} was further used. For spring plants these measurements were not made and rather arbitrarily, the rate of CO_2 production due to maintenance processes found in winter plants, expressed on a protein basis was used as an estimate.

The amount of CO_2 production which remained after subtracting the estimated amount due to maintenance processes (Table 25) was considerable, representing in both types of plant considered 50% of the total amount. If it is explained in terms of protein redistribution 51.4 mg, or 179% of the daily protein accretion would be involved in winter plants, and 193 mg, or 65% in spring plants.

To explain these large amounts of CO_2 production, the following possibilities should be considered:

1. Underestimation of the amount of CO_2 production associated with the formation of different plant compounds
2. Underestimation of the amount of reserves in the plant
3. Important amounts of NO_3^- are reduced in the course of the night

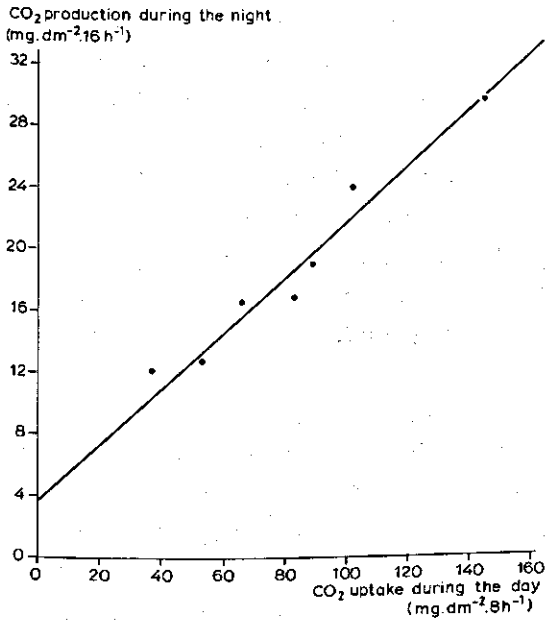

Fig. 26. Relation between CO_2 uptake during the day and CO_2 production during the night in shoots of winter plants. Variations in the amount of CO_2 uptake were obtained by changing irradiance (17-55 $W.m^{-2}$), or CO_2 concentration (300-1500 $\mu l.l^{-1}$).

4. Protein turnover is at least partly controlled by the sugar concentration
5. ATP production is controlled by the sugar concentration and an important fraction of it is hydrolysed without a useful effect (idle respiration).

Possibility 1 If it is assumed as an approximation that the fraction of protein redistribution during the night is equal to the fraction of C incorporation into structural dry weight in that period, the calculated amount of CO_2 production during the night is about 1/3 lower than the observed value, or 50% lower, when referring to the observed value minus the calculated amount of CO_2 production due to maintenance processes.

Although there is still little experimental proof for the correctness of the biochemical approach of Penning de Vries (1974), deviations of this magnitude have not been observed. In the experiments of Penning de Vries & Van Laar(1976) a systematic overestimation of the amount of seedling weight formed from a certain amount of seed reserves could be observed. If this overestimation were entirely due to underestimation of the amount of growth respiration, the 10% deviations found in the seedling weight of *Phaseolus* must be caused by $\pm$ 25% deviations in the amount of respiration (at a growth efficiency of $\pm$ 70%). The 20-30% deviations found in maize were ascribed to leakage of assimilates from the seed, but as the amount of leakage was not determined other factors might have been involved as well. The experimental results obtained with *Arachis*, however, show a very good correspondence with the predicted values.

In his experiments with maize and sunflower, Penning de Vries (1975 a) calculated the slope of the relation between CO_2 uptake in the light and CO_2 production in the subsequent dark period. His calculations clearly showed a tendency to overestimate the amount of CO_2 production due to growth. An important assumption in these calculations, however, was that growth rates in the light and in darkness are equal. My experiments with spring plants, which exhibit an essentially constant rate of CO_2 production in the night, suggested that growth rate, measured as C incorporation in structural dry weight, was much lower at night than in the day.

Possibility 2 To determine the amount of reserves formed in the day, starch and sugar content in the plant have been measured. If besides the amounts determined other reserves were available, the amount of CO_2 production associated with the formation of carbohydrates, lignins and lipids would have been underestimated. This in turn leads to an overestimation of the remaining

fraction of CO_2 production. For spring plants 30-40% more reserves would double the amount of carbon incorporation in structural dry weight and bring the calculated amount of protein redistribution close to that value. In winter plants such shifts would lead to the conclusion that all growth took place during the night. This possibility, however, seems unlikely because there is much evidence in literature that CO_2 assimilated in the light is immediately transformed into structural compounds (e.g. Dickson & Larson, 1975).

Possibility 3 Although possibility 3 is not probable in view of the literature cited in Section 5.1, there was no absolute proof against the occurrence of important amounts of NO_3^- reduction. In the experiment described in Section 5.2, the abundant NO_3^- which is present in most parts of the plants considered may not have been readily accessible. With the assumption that all protein growth during the night was obtained by reduction of NO_3^-, in the same proportion to C incorporation in structural dry weight as during the day, reasonable results could be obtained in the calculations of Table 25.

Possibility 4 The possibility that an important amount of protein turnover is related to growth rather than to plant weight was also mentioned by Penning de Vries (1975b). It is supported by the review of Steward & Bidwell (1962), in which they stated that protein turnover is a concomitant to protein growth and that a high rate of protein synthesis is associated with a high rate of protein turnover and vice versa. If this explains the high rate of CO_2 production, the approach used in this study may serve to estimate the rate of protein turnover in plants, which is a difficult task with other methods (Huffaker & Peterson, 1974).

Possibility 5 Beevers (1970) suggested that idle respiration plays a role in old cells, where "respiration is not so slow as might be expected" (see also Table 12 and 13). Idle respiration of course may explain any difference between observed and calculated rate of CO_2 production and if not predictable, makes these calculations useless.

One of the difficulties in the interpretation of measurements of CO_2 production in shoots, is the lack of knowledge about the processes that take place in the light periods. The roots are in a somewhat different position, in that they contain no photosynthesizing parts and are growing entirely

heterotrophically, so that respiration rate can be monitored continuously.

CO_2 production in the roots, in addition to the processes of maintenance and growth, is also caused by mineral uptake and decarboxylation of organic acids. Rate of CO_2 production due to maintenance was estimated by using the value found in winter plant roots at the end of the dark period. It should be noticed, however, that this value was very high, 0.89 mg CO_2 per mg protein per 24 h, or 8 times higher than the value found in winter plant leaves. In Table 26 contribution of different processes to CO_2 production in the root are given and the total amount calculated in this way is compared with the values measured.

There is a reasonable agreement between the estimated and the measured amount of CO_2 production. CO_2 production due to growth represents a fairly unimportant fraction being only 14% for winter plants and 25% for spring plants, maintenance processes being the main source of CO_2 production. These values are of the order of the differences between calculated and measured amounts of CO_2 production. Although CO_2 production of the root can be 'explained' in terms of growth, maintenance, mineral uptake and decarboxylation of organic acids some facts remain unclear.

The maintenance component, which was estimated from the rate of CO_2 production of winter plant roots at the end of the night, was about 8 times higher than the value found in the leaves. This increased value may be as-

Table 26. Root respiration and its different components, of standard spring and winter plants.

Process	CO_2 production ($mg.plant^{-1}.24\ h^{-1}$)
winter plants	
growth	4.35
mineral uptake	3.16
decarboxylation of org. acids	3.11
maintenance respiration	21.3 +
total calculated respiration	31.9
result from measurements	36
spring plants	
growth	63.0
mineral uptake	30.0
decarboxylation of org. acids	27.0
maintenance respiration	130.4 +
total calculated respiration	250
result from measurements	259

cribed to the activity of micro-organisms living on the roots. In the solution without the roots no significant CO_2 production was measured. Woldendorp (1963) described that an intensive microbial life is present in a thin mucigel layer around the roots. Microbes are known to have very high rates of protein turnover, whereas much energy is required to maintain ion gradients because of the high surface/volume ratio (Penning de Vries, 1975b).

Consequently microbes contribute considerably to the rate of CO_2 production of the roots. If the rate of CO_2 production due to maintenance per g protein is assumed to be the same in the roots as in the leaves, ± 52% and ± 46% of total root respiration may be ascribed to microbial activity in winter and spring plants, respectively. Woldendorp (1963) comparing rates of CO_2 production of sterile and non-sterile pea plant roots, found a reduction of 33% in the sterile plants. He ascribed this difference to microbial activity, arriving thus at a comparable magnitude. It may, however, be questioned whether both plants were comparable in view of their treatment.

The relation between sugar concentration and rate of CO_2 production of the roots is not very clear. Although roots of winter plants reacted drastically to the decreasing sugar supply in the last hours of the night, other factors strongly interfered at other moments. It is possible that respiration of microbes, which are presumably living from the organic excretions of the roots (Rovira, 1969), was somewhat out of phase with the respiration of the roots because of a delay in food supply.

The results obtained with the roots give no further indications about the correctness of the calculated amount of CO_2 production caused by growth, because it represents only a small fraction of the total amount.

The study of individual leaves may elucidate the ideas obtained so far. In fact leaf 1 is very suitable for this purpose because its growth is negligible. CO_2 production therefore must be due exclusively to maintenance processes, protein mobilization and sugar export. Table 27 shows the calculated contribution of different processes and the measured amount of CO_2 production during the night. In this calculation, the carbohydrate reserves are assumed to be equal to the average leaf reserve content, but even doubling this amount leaves an important fraction of CO_2 production unexplained.

Challa & Krupa (in prep.) found that when the first leaf of a standard winter plant was exposed continuously to $^{14}CO_2$ during the day, the $^{14}CO_2$ was exported for only about 50% of the value calculated from the growth analysis. They concluded that an important fraction of the amount of ^{14}C given, mixed with the existing pools, and that the only mobile pool large enough to account

Table 27. Analysis of the CO_2 production of the first leaf of a 5-leaf winter plant.

Process	CO_2 production ($mg.leaf^{-1}.16\ h^{-1}$)
maintenance respiration	2.2
carbohydrate transport	0.9
protein mobilization[1] (24 h value)	0.9
measured CO_2 production	5.7

1. According to Penning de Vries & Van Laar (1976) 1 g protein → 0.767 g amides + 192 g CO_2.

for the observed effect was the protein pool. These observations support the idea that protein turnover is higher than indicated by Penning de Vries (1975b).

It can be finally concluded that calculation of the amount of growth during the night in spring and winter plants from measurements of CO_2 production with the data of Penning de Vries et al., (1974) would lead to severe overestimations. The reason for this discrepancy is not clear. Although changing some of the assumptions may help to explain it, more results are to be expected from a careful study of the relation between protein metabolism and plant growth.

5.4 TIME COURSE OF THE RATE OF CO_2 PRODUCTION IN THE SHOOTS OF WINTER PLANTS

The rate of CO_2 production in the shoots of winter plants during the night plotted against time showed a very pronounced pattern, that has also been described for other plant species; e.g. tomato (Ludwig et al., 1975), clover and sorghum (McCree, 1974). Schematically it may be represented by Fig. 27 in which 3 phases can be distinguished: an initial Phase 'a', which shows an exponential decay, Phase 'b', characterized by an equilibrium situation and Phase 'c', in which CO_2 production decreases exponentially to a new equilibrium value.

The relation between sugar concentration in the leaves and CO_2 production of the shoot is linear (Fig. 25) so that Fig. 27 essentially represents the time course of the sugar content in the leaves. Phase 'a' now can be characterized as a transition between day and night in which sugar content gradually decreases because of an imbalance between supply from starch breakdown and demand for assimilates. In Phase 'b' an equilibrium is maintained until, with the onset of Phase 'c', starvation sets in due to depletion of the starch

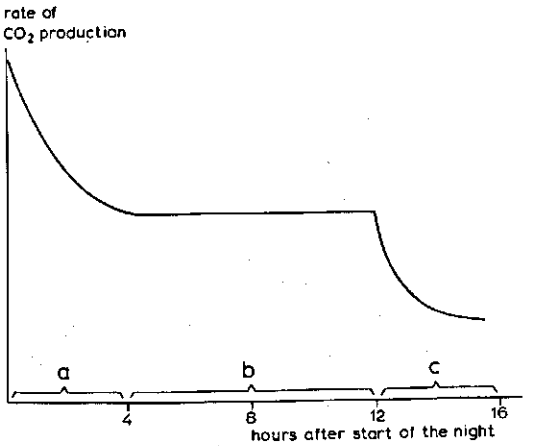

Fig. 27. Schematic representation of CO_2 production of a winter plant shoot during the night, showing the transition Phase 'a', the equilibrium Phase 'b' and the starvation Phase 'c'.

reserves.

Different phases are also characterized by different substrates: in Phase 'a' leaf sugars, whose content has increased in the light, contribute to an important extent to the supply of substrate (Table 16). In this phase little or no carbon is incorporated in structural dry weight, according to Table 22.

In Phase 'b' all carbon substrate is supplied by starch breakdown. Carbon is mainly incorporated in structural dry weight in this phase. This, and protein redistribution processes may also explain the rise in the rate of CO_2 production observed in the youngest leaves (Fig. 18). In Phase 'c' starch breakdown and leaf sugars cannot meet the demand for assimilates and additional respiration substrates are supplied by protein breakdown (Section 5.1). At the end of the night an equilibrium is obtained, in which the rate of protein breakdown equals the rate of CO_2 production.

In spring plants Phase 'c' was not normally observed because of their much shorter night. However CO_2 production rapidly decreased shortly after the normal start of the light period when the plant was kept in darkness. This decrease may be expected from the extrapolation of the curve describing the relation between leaf starch content and time (Fig. 20). In contrast to winter plants, spring plants also incorporate C in structural dry weight in Phase 'a' (Table 22).

Under the circumstances of Phase 'c', a fixation of the *status quo* seems preferable to a progressive process of starvation. Hence a temperature decrease to a level that is just above the limit where damage would occur may be expected to have at least no negative effect on plant growth. Cucumber

plants belong to the group of chilling-sensitive plants (Lyons, 1973), which show damage after exposure to temperatures below 10-12 °C, and consequently this is the minimum temperature which may be applied.

To test this supposition, cucumber plants were cultivated under standard winter plant conditions, except that air temperature was kept at 10 °C for the last 4 hours of the night. This cold treatment was started one day after transplanting the seedlings. Root temperature was kept at 25 °C.

The temperature of 10 °C was already in the chilling range: the plants showed severe wilting phenomena during and shortly after the cold treatment, whereas after restoration, quite in contrast to the normal situation, the position of the leaf blades was almost vertical. During the experiment, the leaves formed a somewhat spherical surface because the growth of the margins was hampered. Both phenomena must have negatively affected light interception of the plants. During the experiment the plants seemed to become hardened to a certain extent and showed less pronounced wilting phenomena. In a second experiment the temperature of the cold treatment was 12 °C which was enough to avoid visible chilling effects. Growth curves for both treatments and that of the standard winter plant are shown in Fig. 28. The 10 °C treatment resulted in a small reduction in growth rate, but the 12 °C treatment enhanced plant growth in comparison with the standard treatment.

In young, exponentially growing plants, the external factors change the net amount of CO_2 uptake over 24 h, but their effect on growth rate may also be mediated by morphological adaptations. To separate both effects, a growth analysis was carried out estimating the net assimilation rate by

$$\frac{W_2-W_1}{A_2-A_1} \cdot \frac{\ln A_2-\ln A_1}{t_2-t_1}$$

and average leaf ratio by

$$\frac{1}{2}\left(\frac{A_1}{W_1} + \frac{A_2}{W_2}\right)$$

where W = plant weight
A = leaf area
t = harvesting time.

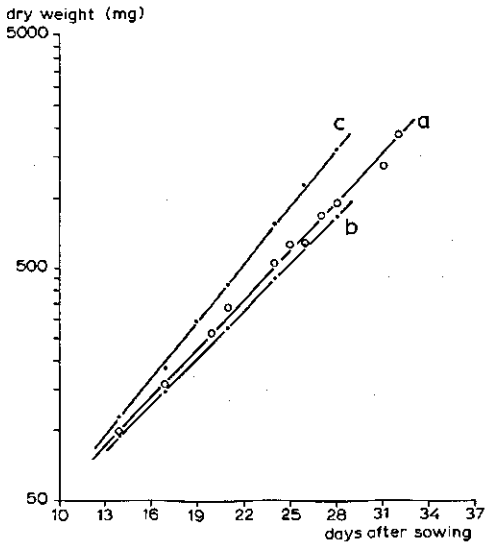

Fig. 28. Dry weight of winter plants against time. a. plants growing under standard conditions, b. under standard conditions but for the last 4 hours of the night at 10 °C, c. the same as b. but at 12 °C.

The results are presented in Table 28, together with a comparison of some important plant characteristics at the end of the 26-day growing period.

Table 28. Comparison of growth analysis data over the last 14 days of a 26-day growing period of winter plants and some plant characteristics at the end of the growing period. Plants were grown either under standard conditions or under otherwise identical conditions with an air temperature of 12 °C or 10 °C during the last 4 hours of the night.

Treatment	Standard	10 °C	12 °C
growth characteristics:			
(day 12-26)			
RGR ($g.g^{-1}.day^{-1}$)	0.157	0.154	0.193
NAR ($mg.dm^{-2}.day^{-1}$)	27.5	30.4	39.6
$\overline{LAR}$ ($dm^2.g^{-1}$)	5.50	4.65	4.80
plant characteristics:			
(day 26)			
LAR ($dm^2.g^{-1}$)	5.71	5.17	5.02
SLA ($dm^2.g^{-1}$)	8.16	7.46	7.33
LWR ($g.g^{-1}$)	0.70	0.69	0.69
RWR ($g.g^{-1}$)	0.082	0.071	0.079

LAR = leaf area ratio
SLA = specific leaf area
LWR = leaf weight ratio
RWR = root weight ratio
RGR = relative growth rate
NAR = net assimilation rate
$\overline{LAR}$ = average leaf area ratio

Subjecting winter plants to a cold treatment of 12 °C for the last four hours of the night leads to an increased relative growth rate, caused by a much enhanced net assimilation rate, which cannot be explained by a decrease in the rate of night respiration alone. Therefore it must be concluded that carbohydrate depletion gives rise to disadvantageous processes and that these processes can be suppressed at least partly by decreasing the air temperature.

It will be shown in Section 5.5 that the starvation phase starts after 11-12 h of darkness, independent of the growing conditions. Thus it may be expected that reduction of air temperature after 12 h night to a level of 12 °C will help to save energy and to improve crop productivity in glass house culture in winter time.

5.5 SOME CONSIDERATIONS ABOUT LEAF-STARCH BREAKDOWN

Starch reserves play an important role in determining the amount and time course of CO_2 production of the shoot. In the first place the majority of the carbohydrate reserves are lost by respiration (about 70%). In the second place, the onset of Phase 'c' (Section 5.4), in which processes that negatively affect plant growth predominate, is determined by the moment when starch reserves are depleted. It is therefore worthwhile to consider more closely the control of starch breakdown.

The decrease in leaf starch content in both spring and winter plants is approximately linear in time and, as may be expected by extrapolation, depletion occurs after about 11-12 h (Fig. 20). As these plants were growing under quite different conditions of assimilate supply, starch breakdown could be under the control of an endogeneous rhythm, synchronized by the light/dark transition, such as has been demonstrated in the photosynthesizing unicellulars *Chlamydomonas* (Queiroz, 1974) and *Acetabulara* (Van den Driessche, 1970). Fig. 29 shows the diurnal course of starch content in the leaves of a winter plant, growing under high CO_2 concentration ($\pm$ 1500 $\mu l.l^{-1}$). Even though the content is 50% higher at the end of the day, depletion again is observed after 12 h darkness. Figure 30 gives the time course of the rate of CO_2 production in the shoots of standard winter plants, which received different treatments on the preceding day. In almost all cases, the onset of Phase 'c', which is caused by depletion of starch reserves (Section 5.4) is observed after 11-12 h. Decreasing the night temperature from 25 ° to 20 °C did not affect this moment either (Fig. 30). Ludwig et al. (1975) and McCree (1974) found that the onset of Phase 'c' appeared to be independent of irradiance and CO_2

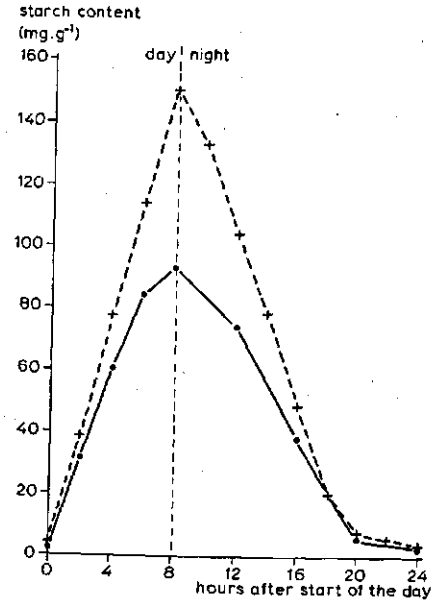

Fig. 29. Diurnal course of leaf starch content of winter plants, cultivated under a raised CO_2 concentration of 1700 $\mu l.l^{-1}$ (+—+), compared with the standard winter plant (●—●). Contents are expressed on basis of starch and sugar free dry weight.

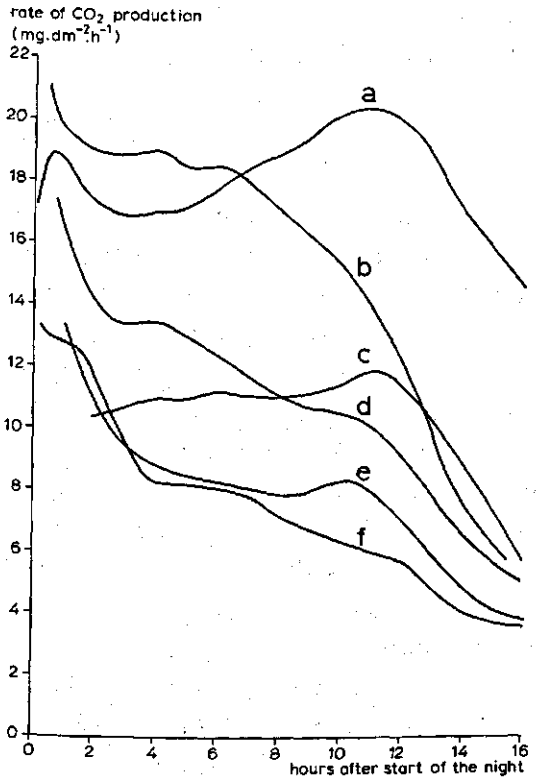

Fig. 30. Time course of the rate of CO_2 production of winter plant shoots cultivated under standard conditions, but receiving during the preceding day or during the night a different irradiance, CO_2 concentration, or temperature.
a. 55 $W.m^{-2}$, 1500 $\mu l.l^{-1}$ and 30 °C during the day, b. 45 $W.m^{-2}$ during the day, c. 20 °C during the night, d. 1500 $\mu l.l^{-1}$ during the day, e. standard treatment, f. 17 $W.m^{-2}$ during the day.

concentration during the preceding day, and of night temperature. More research will be needed to investigate the underlaying mechanisms and to test the generality of this phenomenon, but in the cases described here it seems to be well established.

5.6 DAILY COURSE OF THE RATE OF CO_2 UPTAKE

Under constant irradiance, leaves of both spring and winter plants showed variations in rate of CO_2 uptake. At the onset of the day, a certain time lag was observed between switching on the light and the equilibrium value of CO_2 uptake. Furthermore a decrease in the rate of CO_2 uptake was observed after about 7 h light in leaves of spring and winter plants, causing a reduction of about 10% of the expected total daily amount of CO_2 uptake in spring plants. Among the factors which may be responsible for such variations are stomatal resistance, dark and photo respiration, and the ability of cells to fixate CO_2.

The time required for photosynthesis to adapt to the light at the start of the day is a generally observed phenomenon that is usually ascribed to building up of certain compounds (e.g. enzymes, substrates), which play a role in CO_2 assimilation and which decrease in concentration in darkness (Walker, 1972).

An important decrease in the rate of CO_2 uptake was observed after $\pm$ 7 h of light, and this decrease was accompained by stomatal closure. Quite similar phenomena were observed by Upmeyer & Koller (1973) working with soya bean leaves. This coincidence, however, does not necessarily imply that stomatal closure actual causes the decrease. Often stomata control the CO_2 concentration of the substomatal cavity, and then stomatal closure may be the result rather than the cause of a reduced rate of CO_2 uptake (Goudriaan & Van Laar, 1976; Raschke, 1975). Because the stomata of the cucumber plants used in these experiments did not respond to changes in external CO_2 concentration, stomatal closure must have been the primary cause of the decrease. The reason for this stomatal closure is not clear. Stomata may close due to water shortage, or unknown internal mechanisms may be involved.

Following the water content of first leaves of spring and winter plants in the course of the day by β-gauging (Fig. 31) shows that indeed water content varied in course of time. Interpretation of the measurements was obscured by the fast growth rate exhibited by the leaf of the spring plant. If rate of increase in fresh weight is assumed to be constant throughout the day,

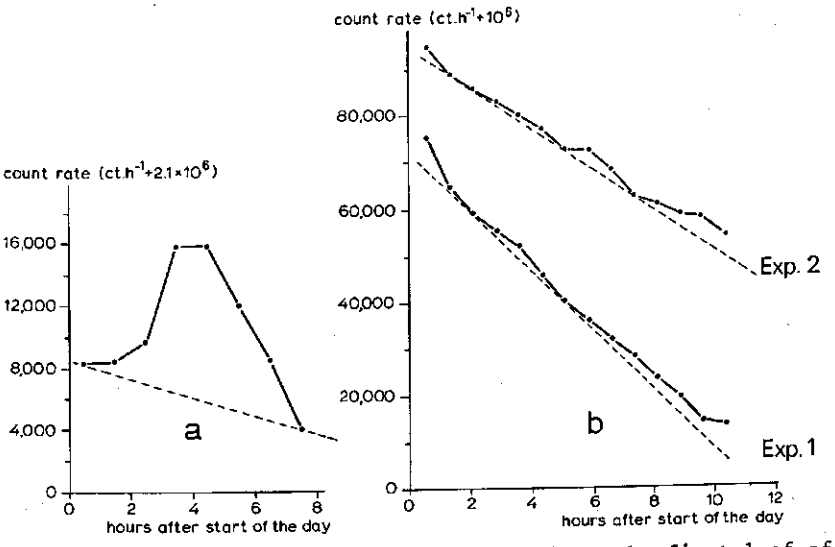

Fig. 31. Count rate measured with a β-gauge on the first leaf of a spring (b) or winter plant (a), growing under standard conditions in the phytotron. If growth rate during the day is constant, deviations from the straight line represent variations in water content.

deviations may be interpreted as changes in water content. Both spring and winter plants showed a maximum water shortage between 4 and 5 h after the start of the day, and this moment coincided with the first signs of stomatal closure as revealed by the transpiration measurements (Section 4.1). In spring plants during Experiment 1, water balance was not restored even though stomata closed after 8 hours of light. During Experiment 2 on the same plant, stomatal closure was probably followed by a recovery of the water balance and subsequent reopening of the stomata as can be observed in some of the experiments of Section 4.1. It should be noticed that moments of maximum water shortage coincided with maxima in leaf sugar concentration (Section 4.3), which is a commonly observed phenomenon (Mothes, 1956). It should be stressed at this point that the changes in water content observed are fluctuations probably occuring in every plant that is exposed to light, and that no visible signs of water shortage could be observed.

Parsons & Kramer (1974) and Shirazi et al. (1975) observed a diurnal rhythm in the resistance of cotton roots to water. Thus I tried to exclude the effect of root resistance. Unfortunately cucumber plants, on cutting, excrete so much phloem exudate that the xylem is readily plugged. To avoid this problem the root system was kept for a few seconds in hot water (80 °C), which should greatly increase its permeability (Slatyer, 1967). Indeed stomata

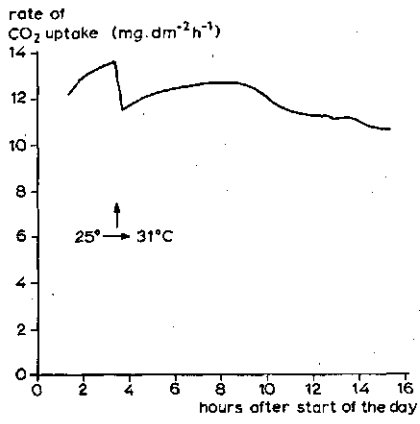

Fig. 32. Daily course of the rate of CO_2 uptake of leaf 3 of a spring plant under otherwise standard conditions, but air temperature increased from 25 °C to 31 °C.

started to reopen, but within 20 min the situation reversed and stomata closed again. Removal of all leaves, except one which should reduce the rate of transpiration by a factor 5, also resulted only in a short-term effect on stomatal opening. Hence it is unlikely that stomatal closure is controlled directly by the water balance.

Raschke (1975) suggested that plants that did not control the internal CO_2 concentration of their leaves at first may be induced to do so, for example, by water shortage or abscissic acid content changes. If this were so, stomata should be responsive to changes of CO_2 concentration, when they are in the closed situation. Experiments showed that the stomata were not responsive in this situation.

When the start of the day was shifted a few hours in advance or backwards, the moment of stomatal closure was shifted accordingly. This phenomenon may be interpreted as a synchronization with an internal rhythm (Sweeney, 1969; Haberman, 1974; Hopmans, 1971; Pallas et al., 1974), but accumulation of some product formed in the light could also account for this effect (Neales & Incoll, 1968).

Some evidence for this last possibility was found in an experiment in which air temperature was increased from 25 to 31 °C (Fig. 32). Although a small decrease in the rate of CO_2 uptake and transpiration was observed, it was much less pronounced than under normal conditions. The possibility that the stomata closed partially due to the change in temperature, however, has not been investigated.

Summary

In the research for improvement of glasshouse climate control, simulation models may be of great value, provided that the short-term simulation of plant growth reactions can be sufficiently refined. To develop such models the diurnal course of carbon dioxide exchange, transpiration and reserve carbohydrate content was measured on young cucumber plants.

The development of the necessary equipment resulted in a new system for the preparation of gas mixtures with various constant CO_2 concentrations by precision flow controllers. The differential water vapour analyser was calibrated applying a principle that is new to the plant physiological literature. A known water vapour pressure difference was created by condensation of humid air in two vessels, which were at the same temperature, but at different pressure. After getting rid of the pressure difference, the resulting difference in water vapour pressure could be calculated.

Measurements were made on organs of 5-leaf cucumber plants, cultivated in climate rooms. For comparison two types of plant were studied: winter plants, growing for short days (8 h) at low irradiance (30 $W.m^{-2}$) and spring plants growing for relatively long days (14 h) at a high irradiance (80 $W.m^{-2}$). The results of these measurements are presented in Chapter 4, which is rather descriptive. Most remarkable in these results is the decrease in the rate of CO_2 uptake by the spring plant about 8 hours after the start of the day, the strong decrease in the rate of CO_2 production of the winter plant after about 12 hours of darkness and the very regular pattern of the course of the starch content in the leaves of both types of plant.

Most important results were obtained, however, in comparing the results of different measurements. In winter plants 43% of the daily amount of CO_2 uptake was used for the formation of carbohydrate reserves. In spring plants this fraction was only 23%, in spite of higher irradiance and a longer day. The efficiency of carbon incorporation from the daily CO_2 uptake in structural dry weight was 62% in winter plants and 77% in spring plants.

The conversion of reserve carbohydrates to structural dry weight during the night was much less efficient: 28% in winter plants and 32% in spring

plants. The majority of these reserves were lost by respiration during the night.

If it is assumed that NO_3^- is reduced and organic acids and proteins are formed during the day, the carbon incorporated in the remaining structural dry weight during the day and the night can be calculated from the carbon balance. In winter plants 44% and in spring plants 22% of this organic matter was formed during the night. This implies that the average rate of formation during the night was about half of that during the day. Likewise, carbon balances were calculated for shorter intervals. In this way it was deduced that in winter plants carbon incorporation in structural compounds strongly increased during the day. During the night incorporation mainly took place between 4 and 12 hours after the start of the night. At the end of the night most probably proteins were broken down. In spring plants carbon incorporation in structural compounds varied little during the night. During the day, however, a reduction was observed in these plants after about 8 hours of light, which is likely to be related to a reduction in the rate of CO_2 uptake.

Penning de Vries calculated on a theoretical basis the relation between CO_2 production and formation of different plant components. This work provided a base for my study on the usefulness of CO_2 production of shoot and root as a measure for growth rate. For the roots a reasonable agreement was found between predicted and measured CO_2 production. However, CO_2 production due to growth only contribute 14% in winter plants and 25% in spring plants, mainly as a result of the very high rate of CO_2 production due to maintenance. In the shoot the calculated value considerably underestimated the amount of CO_2 production. The cause of this discrepancy is not clear, but there are some indications that protein turnover might play a role in explaining this difference.

The time course of CO_2 production of the winter plant reflected the time course of the leaf sugar content. Fluctuations in the CO_2 production in the shoot and leaf sugar content were determined to a large extent by the main substrate for respiration, successively sugar, starch and protein. Protein breakdown which takes place after 12 hours darkness in winter plants is a harmful process. This was shown in an experiment in which the air temperature during this period was decreased to 12 ^{o}C. The treatment resulted in an increased growth, which has to be explained by a reduction of the damage that normally occurs in this starvation phase. This observation is of great value in climate control programmes in glasshouses, particularly in connection with the problems of saving energy.

The moment at which proteins are drawn on as a respiration substrate is connected with the moment of starch depletion. In a number of experiments in which plants took up different amounts of CO_2 during the day, the moment of starch depletion was always the same, indicating that starch breakdown was 'preprogrammed'.

The reduction of the rate of CO_2 uptake after about 8 hours of light in spring plants was caused by stomatal closure, which could not be explained. It is, however clear, that neither the water balance nor internal leaf CO_2 concentrations were concerned with stomatal closure but perhaps accumulation of photosynthetic products did play a role.

Samenvatting

Optimalisering van het kasklimaat wordt bereikt door onder gegeven omstandigheden, binnen de grenzen van het technisch mogelijke, een dusdanig compromis te vinden tussen produktiesnelheid, teeltkundige eisen en de eraan verbonden kosten, dat voor de tuinder het maximaal economisch rendement wordt verkregen.

De snelheid waarmee met name de factor instraling varieërt, vereist een relatief snelle aanpassing van de kasklimaatregeling en daarmee samenhangend, de mogelijkheid om het effect van deze regeling op de produktiesnelheid op korte termijn te meten. Bij nadere beschouwing blijkt evenwel, dat verschillende groeiprocessen binnen een etmaal een verschillende dynamiek volgen, zodat de benadering van het groeiproces als een eenheid in dit verband niet zinvol is.

Een evaluatie van de effecten van de klimaatregeling op de produktiesnelheid zou mogelijk zijn, door bestudering van de reacties van de individuele groeiprocessen en door, rekening houdende met hun onderlinge interacties, deze in te bouwen in simulatie modellen, zoals die de laatste jaren in Wageningen zijn ontwikkeld. Een verfijning van deze modellen is hierbij echter noodzakelijk, om de, voor deze korte termijn benadering, vereiste detaillering in het dagelijkse verloop van de groeiprocessen te kunnen bereiken.

In het kader van de verfijning van deze groeimodellen ten behoeve van de klimaatregeling in kassen werd een onderzoek verricht naar het dagelijkse verloop van CO_2-gaswisseling, transpiratiesnelheid en koolhydraatreservegehalte van jonge komkommerplanten.

De ontwikkeling van de, voor dit onderzoek, benodigde apparatuur mondde uit in een nieuw systeem voor de vervaardiging van gasmengsels met, door middel van precisie flowregelaars, instelbare CO_2-concentraties. Voor de ijking van de differentiële waterdampanalysator werd gebruik gemaakt van een, in de plantenfysiologische literatuur, nieuwe principe. Hierbij wordt een bekend waterdampspanningsverschil gecreëerd, door vochtige lucht in twee vaatjes bij gelijke temperatuur te laten condenseren bij een bekend drukverschil. Na nivellering van het drukverschil kan het dampspanningsverschil berekend worden.

De metingen werden verricht aan organen van 5-blads komkommerplanten, welke werden opgekweekt in klimaatcellen. Ter vergelijking werden twee typen planten bestudeerd: Winterplanten, groeiend bij een korte dag (8 uur) en lage instraling (30 $W.m^{-2}$) en voorjaarsplanten groeiend bij een relatief lange dag (14 uur) en een hoger stralingsniveau (80 $W.m^{-2}$). De resultaten van de metingen van het dagelijkse verloop zijn vastgelegd in hoofdstuk 4, dat een sterk descriptief karakter draagt. Meest opmerkelijk in deze resultaten is de afname van de CO_2-opnamesnelheid bij de voorjaarsplant $\pm$ 8 uur na het begin van de dag, de sterke afname van de CO_2-produktie bij de winterplant $\pm$ 12 uur na het begin van de nacht en het regelmatige verloop van het zetmeelgehalte in de bladeren van beide typen planten.

De belangrijkste resultaten werden evenwel verkregen, door onderlinge vergelijking van de verschillende metingen. Bij winterplanten wordt tijdens de dag 43% van de opgenomen hoeveelheid CO_2 gebruikt voor de vorming van koolhydraatreserves. Bij voorjaarsplanten bedraagt deze fractie slechts 23%, ondanks de hogere instraling en grotere daglengte. De efficiëntie waarmee de door de spruit tijdens de dag opgenomen CO_2 wordt omgezet in structurele droge stof bedraagt 62% bij winterplanten en 77% bij voorjaarsplanten. De efficiëntie waarmee tijdens de dag gevormde koolhydraatreserves tijdens de nacht worden omgezet in structurele droge stof ligt echter beduidend lager: 28% bij winterplanten en 32% bij voorjaarsplanten. Deze reserves worden dus voor het grootste gedeelte tijdens de nacht verademd.

Indien wordt aangenomen, dat de reductie van NO_3^- en de vorming van eiwit en organische zuren uitsluitend tijdens de dag geschieden, kan via de koolstofbalans de koolstofinbouw in de overige organische stof tijdens de dag en de nacht worden berekend. Zo bleek bij winterplanten 44% en bij voorjaarsplanten 22% van deze organische stof tijdens de nacht gevormd te worden. Dit betekent, dat de gemiddelde synthesesnelheid ervan tijdens de nacht slechts ongeveer de helft is van de snelheid tijdens de dag.

Soortgelijke koolstofbalansen zijn ook opgesteld voor kortere intervallen. Hierbij bleek, dat de koolstofinbouw in structurele droge stof in de loop van de dag sterk toeneemt bij winterplanten. 's Nachts vindt inbouw vooral plaats tussen 4 en 12 uur na het begin van de nacht. Aan het einde van de nacht worden zeer waarschijnlijk eiwitten afgebroken. Bij voorjaarsplanten is de koolstofinbouwsnelheid in structurele droge stof min of meer constant tijdens de nacht. Gedurende de dag kan een afname worden geconstateerd na $\pm$ 8 uur, welke verband lijkt te houden met een terugloop van de CO_2-opname.

De bruikbaarheid werd onderzocht van de CO_2-produktie van de spruit en

van de wortel als maat voor de groeisnelheid. De mogelijkheid hiertoe werd geschapen door het werk van Penning de Vries, die het verband tussen CO_2-produktie en vorming van verschillende plantbestanddelen langs theoretische weg berekende. Bij de wortel werd een redelijke overeenkomst gevonden tussen berekende en gemeten ademhaling. De groeicomponent van de ademhaling was echter slechts 14% van het totaal bij winterplanten en 25% bij voorjaarsplanten, voornamelijk als gevolg van de zeer hoge onderhoudsademhaling. Bij de spruit is de berekende ademhaling in beide typen planten belangrijk lager dan de gemeten waarde. Waaraan deze discrepantie moet worden toegeschreven is niet duidelijk, maar wel zijn er aanwijzingen, dat eiwitturnover hierbij een rol zou kunnen spelen.

Het verloop van de nachtademhaling van de winterplantspruit is een weerspiegeling van het verloop van het bladsuikergehalte. Fluctuaties in spruit CO_2-produktie en in bladsuikergehalte worden in grote mate bepaald door het hoofdsubstraat waaruit de ademhaling wordt gevoed, namelijk achtereenvolgens suikers, zetmeel en eiwitten. De afbraak van eiwitten na 12 uur duisternis bij winterplanten is een schadelijk proces. Dit blijkt uit de resultaten van een experiment waarbij gedurende deze periode de luchttemperatuur werd verlaagd dot 12 ^{o}C. De behandeling leidde tot een stimulering van de groei, hetgeen grotendeels verklaard moet worden door een vermindering van de schade, die normaal optreedt in deze hongerfase. Deze waarneming is van groot belang voor de regeling van het kasklimaat, vooral in verband met de energiebesparingsproblematiek.

Het moment waarop eiwitten worden aangesproken als ademhalingssubstraat hangt samen met het moment waarop de zetmeelreserves opraken. In een aantal gevallen, waarbij de CO_2-opname tijdens de dag sterk werd gevariëerd, bleek dit moment steeds na het zelfde aantal uren duisternis op te treden, hetgeen er op duidt, dat de zetmeelafbraak 'geprogrammeerd' in de tijd verloopt.

De terugloop van de CO_2-opnamesnelheid bij voorjaarsplanten na $\pm$ 8 uur licht, bleek veroorzaakt te worden door huidmondjessluiting. Voor deze sluiting kon geen oorzaak gevonden worden. Wel is duidelijk, dat watergebruik en interne koolzuurconcentratie in het blad hierbij geen rol spelen, maar mogelijk wel de ophoping van bepaalde fotosyntheseprodukten.

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