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Rates of Respiration and of Increase in Structural Dry Matter in Young Wheat, Ryegrass and Maize Plants in Relation to Temperature, to Water Stress and to Their Sugar Content

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ABSTRACT

A method to determine the rate of conversion of reserve materials into structural dry matter in living, whole plants is presented. It is based on a brief measurement of plant respiration. The rate of increase in structural dry matter and of decrease of reserve materials is calculated by subtracting the maintenance respiration component from the total respiration. The remainder, the growth respiration rate, is multiplied by a factor that is derived from the biochemical composition of the structural dry matter formed.

This method is applied to determine the relations of the rate of conversion of reserve material into structural dry matter to temperature, water stress and the level of reserve carbohydrates in plants of three species.

The weakest part of the method is in the determination of the rate of maintenance respiration. Consequences of different assumptions concerning the rate of adjustment of this respiration component to modified environmental conditions are discussed.

Keywords: *Lolium perenne* L., *Triticum aestivum* L., *Zea mays* L., ryegrass, wheat, maize, respiration, maintenance respiration, water stress, sugar content, structured dry matter.

INTRODUCTION

Students of photosynthesis have accumulated much knowledge about the rate of production of sugars in plants. These sugars, in general, are either combusted in cells to provide energy for maintenance and transport processes, or are converted into more permanent components. Together, such components may be called 'structural dry matter' (SDM), and include cellulose, protein, lignin and fat. The short-lived sugars may be described as 'reserve carbohydrates' (RC). Conversion of RC into SDM occurs typically in heterotrophic cells, and in this paper, conversion of RC into SDM will be referred to as heterotrophic growth. It comprises growth of protoplasma and of cell walls and occurs throughout the day and night.

Heterotrophic growth approximates to net photosynthesis over periods of a few days, and can thus be determined by gas exchange measurements over such a time span. These measurements average out the effect of internal regulation and adaptation, even in a constant environment, and do not increase much our understanding of the dynamics of heterotrophic growth during the course of a day. Such knowledge, though, is indispensable for manipulation of the plant environment for optimalization of plant production in greenhouses (Challa, 1976), and will contribute in a general sense to our understanding of productivity of crops.

Heterotrophic growth may be observed by harvesting groups of plants only hours

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apart and analysing them to detect by how much the amount of SDM changed. This direct measurement of growth involved necessarily large batches of plants to reduce the statistical variation to an acceptable level, and seems therefore unpractical. Moreover, this procedure still produces a rate of growth averaged over some hours during which it may have changed considerably.

Leaf elongation is partially based on growth of protoplasm and cell walls and partially on water and solute intake into vacuoles. The rate of leaf elongation is therefore not necessarily correlated with the rate of heterotrophic growth. As it is a simple measurement, however, the rate of leaf elongation was determined in most of the experiments described below.

The measurement of plant respiration seems to provide a more suitable means to determine the rate of heterotrophic growth, since the ratio of the SDM and the CO_2 formed is predictable: in the experiments described below, 0·47 g CO_2 is released as a result of the synthesis of 1·00 g of SDM, as will be shown. The CO_2 released due to heterotrophic growth is called growth respiration. Growth respiration equals total respiration minus the maintenance respiration component in well-illuminated and well-nourished plants. The rate of maintenance respiration can be approximated in a non-growing condition, or can be estimated from the literature (Penning de Vries, 1975*a*; Ryle, Cobby and Powell, 1976). Unfortunately, maintenance respiration is still a little known subject.

For the purpose of calculating the ratio of 0·47, the biochemical composition of the young plants used in these experiments was determined: per g SDM, they consist of 0·53 g structural carbohydrates (cellulose, hemicellulose) plus 0·22 g nitrogenous compounds (proteins, amino acids, nucleic acids) plus 0·08 g lignin plus 0·05 g organic acids plus 0·02 g fats and oils plus 0·10 g minerals. Deviations of this composition by individual species were unimportant. The formation of SDM with this composition from RC only can be described, in its simplest form, by

$$1{\cdot}39 \text{ g RC} \rightarrow 1{\cdot}00 \text{ g SDM} + 0{\cdot}68 \text{ g } CO_2 \qquad (1)$$

(Penning de Vries, Brunsting and Van Laar, 1974. Note that this equation seems imbalanced, since minerals, nitrate, oxygen and water have been omitted in the presentation. RC has been taken to consist of oligo- and polysaccharides). However, the growing cells are supplied with amino acids in addition to RC. Like production of RC, all reduction of nitrate and formation of amino acids occurs only during photosynthesis in the experimental conditions used. If it is supposed that the biochemical composition of the SDM increment does not change immediately as a result of a suddenly introduced dark period, eqn (1) has to be replaced by

$$1{\cdot}02 \text{ g RC} + 0{\cdot}27 \text{ g amino acids} \rightarrow 1{\cdot}00 \text{ g SDM} + 0{\cdot}47 \text{ g } CO_2. \qquad (2)$$

This equation was derived by following a procedure described earlier (Penning de Vries, 1975*b*). There is some experimental evidence that the terms of eqn (2) are independent of temperature (Penning de Vries, 1976; Ryle *et al.*, 1976). On theoretical grounds it is expected that neither environmental nor internal factors modify the eqns (1) and (2) (Penning de Vries *et al.*, 1974).

MATERIALS AND METHODS

Seeds of maize (*Zea mays* L. var. Pioneer) and of wheat (*Triticum aestivum* L. var. opal) were germinated on moistened perlite at 20 °C. After 7 days, seedlings were transferred to 1 l pots containing a nutrient solution and placed in a climate room (Alberda, 1958). Light intensity at plant level was about 0·008 J cm^{-2} s^{-1} for 17 h per day. The maize plants grew here for 7 days, wheat plants for 14 days. Vegetative tetraploid perennial

ryegrass (*Lolium perenne* L.) tillers were cultivated in groups of 5 sprouts on similar pots for about 4 weeks. After these periods, plants and groups of grass tillers weighed 0·5–2 g dry matter each, and were transferred to another climate room. As a standard treatment, plants were illuminated for 24 h at about 0·025 J cm^{-2} s^{-1} with HPL lamps and used immediately afterwards. In all pretreatments the nutrient solution was renewed as often as required to keep sufficient nitrate in the root medium. During all measurements the temperature was 25 ± 1 °C, there was no water stress, and the substrate level in the plants was at a medium level, except in those experiments aimed at variations in these factors. The measurements lasted 1–1·5 h and were executed in darkness.

The measurement of the respiration rate of whole plants in an enclosure was essentially done by continuous registration of the CO_2 concentration of air entering and leaving the enclosure. A mobile laboratory described by Louwerse and Eikhoudt (1975) was used, both air conditioning units and both enclosures being put up in a glasshouse in winter time. Intact plants, with a total d. wt of 10–20 g, were carefully fixed in holes in a perspex plate, which was laid on an open perspex box of 30 × 30 × 8 cm. More than 75 per cent of the roots were in a shallow layer of water with 3 m-mol l^{-1} $CaSO_4$ and some H_2SO_4 to bring its pH between 4 and 5. This solution was prepared more than 24 h before use to expel all dissolved CO_2. It was found that this solution did not act as a source or sink of CO_2 so that respiration of roots was part of the total respiration. Figure 1 is a schematic drawing of the enclosure. The volume of the enclosure was about 125 l, and the flow of air passing through was about 500 l h^{-1}. The respiration rate was determined after about 1 h, when the disturbance of the CO_2 level during initiation of the experiment had disappeared. Immediately after achieving this state, plants were removed from the enclosure, dried, weighed, ground and subjected to chemical analysis. In Figs 2–13, respiration is expressed in mg CO_2 g^{-1} SDM h^{-1}; SDM is used rather than total d. wt (SDM + RC) to avoid effects of 'dilution' by RC present in variable amounts.

Each group of plants was analysed for its total water soluble carbohydrates (TSC) according to the method described by Alberda (1965). The complete biochemical composition of the plants was determined in a few samples only. To avoid confusion in experiments during which the TSC content changes, TSC levels are indicated in mg TSC g^{-1} SDM rather than in percentages.

Leaf elongation was measured by recording the length of a leaf at 0·5, 1 and 1·5 h after initiation of the experiment. One healthy growing leaf was selected for this purpose of about half the size of the last full-grown leaf. The measurement was made by observing the displacement of the leaf tip along a calibrated scale, using a fixed magnifying glass outside the enclosure. The leaf was led between the scale and a transparent plate by a weight of 10 g on a string over a pulley (Fig. 1). This simple method proved to be very satisfactory, insensitive to air turbulence and accurate to ± 0·1 mm.

In experiments in which different levels of substrates were required, plants were illuminated with HPL lamps for 0 up to 50 h continuously, while others were given up to 24 h of darkness. Different temperatures were obtained by heating or cooling the circulating air. Water stress was induced in the plants by immersing the roots in a $CaSO_4$ solution in which polyethylene glycol 6000 (PEG) or mannitol was dissolved. The solution was prepared more than 24 h before use. From the observation, by the same mobile laboratory, that the rate of transpiration was reduced to a new level within 15 min, it was deduced that the plants equilibriated with the solution water potential very quickly.

RESULTS

Responses to temperature

Figures 2–4 present the observed rates of respiration and elongation at temperatures from 5 to 40 °C in wheat, ryegrass and maize. The rate of maintenance respiration at

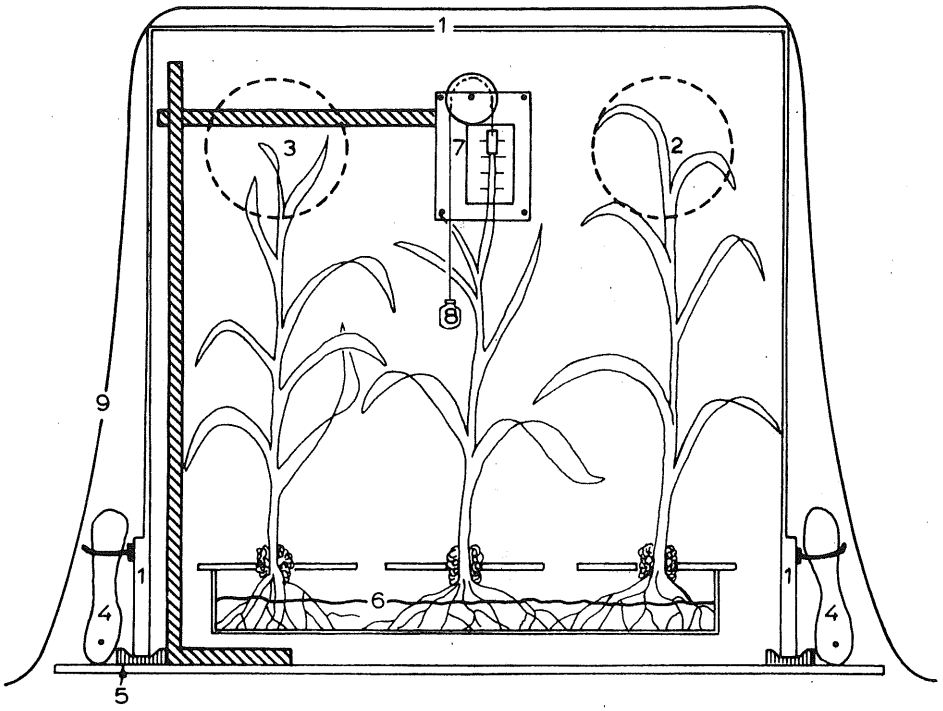

FIG. 1. A diagrammatic cross-section of the enclosure with maize plants. In the back of the cubic perspex chamber (1) is an air inlet (2) and outlet (3). The chamber is fixed to a platform by rubber clamps (4). Rubber foam (5) seals the joint. The perspex container (6), half filled with a $CaSO_4$ solution, is covered by a lid with many holes. Plants are held in some of these holes with soft rubber foam. A scale for measuring leaf length (7) is fixed to a support. About 5 cm of one leaf is led between the scale and a perspex plate to avoid fluttering in the turbulent air, and held upright by a 10 g weight (8) on a string via a pulley. The chamber was darkened by a sheet of black plastic (9).

25 °C will be derived below, and it is assumed to double per 10 °C temperature increase (Penning de Vries, 1975*a*; Ryle *et al.*, 1976). The calculated maintenance respiration is plotted from the temperature axis downwards. The vertical distance from this curve to the experimental data represents the observed rate of respiration, so that the curve above the temperature axis reflects the response of growth respiration to temperature. According to eqn (2), multiplying this rate by 2·13 gives the response of growth rate to temperature. A growth rate of 3 mg g^{-1} SDM h^{-1} corresponds with a relative growth rate of ($1{\cdot}003^{24}$ =) 0·07 g SDM g^{-1} SDM day^{-1}, 6 mg with 0·15 g g^{-1} day^{-1}, 12 mg with 0·33 g g^{-1} and 20 mg with 0·61 g g^{-1} day^{-1}.

The relations of growth respiration to temperature in Figs 2–4 are clear and need no comment. It is not known what increased scatter of the observations occurred in the 35 and 40 °C experiments.

The rate of leaf elongation is also presented in Figs 2–4. This rate reacts more to temperature than does the rate of heterotrophic growth, and reaches a maximum in wheat and ryegrass at temperatures 5–10 °C lower than the growth rate. In maize, both curves follow almost the same pattern. It is expected that these rates have realistic absolute values, since the rates in Fig. 4 compare very well with those found by Gallagher

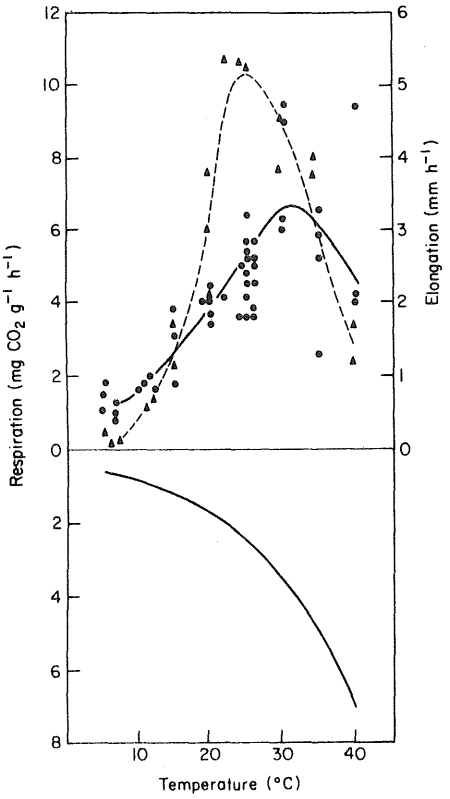

Fig. 2.

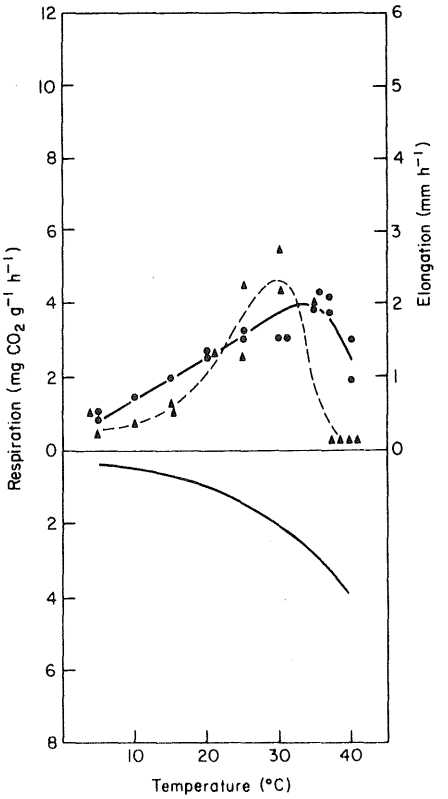

Fig. 3.

FIG. 2. Plant respiration (●, in mg CO_2 g^{-1} SDM h^{-1}) and leaf elongation (▲, in mm h^{-1}) versus temperature in wheat. The calculated rate of maintenance respiration is plotted downwards. The vertical distance between a data point and this line represents the measured rate of respiration. The distance from the data points to the temperature axis represents growth respiration. The rate of heterotrophic growth (mg SDM g^{-1} SDM h^{-1}) is found by multiplying the growth respiration rate by 2·13. Curves through the data points are fitted by eye.

FIG. 3. Plant respiration and leaf elongation versus temperature in ryegrass. For further explanation see Fig. 2.

and Lof (1971). They measured frequently lengths of all leaves of maize plants growing undisturbed under similar conditions until maturity, and found rates of 0·9 mm h^{-1} at 15 °C and 2·5 mm h^{-1} at 20 °C, and 3·5 mm h^{-1} at 25 °C. These rates were constant during much of the growing period of a leaf.

It is interesting to compare the response of growth rate to temperature with the response of photosynthesis. For this purpose, unpublished measurements by Alberda (pers. comm.) on mature leaves of similar plants were used. These indicate that the temperature for maximum photosynthesis in wheat is about 10 °C below its optimum for maximum growth. This holds also for ryegrass, although its rate of photosynthesis was almost constant from 12 to 25 °C. Photosynthesis of maize goes up until 30 or 35 °C, and was not measured at higher temperatures. These observations suggest that the sugar content in wheat and ryegrass plants grown in high temperature environments, and in maize plants to a smaller extent, will often be lower than that in cool environments. This has been observed in ryegrass by Alberda (1965).

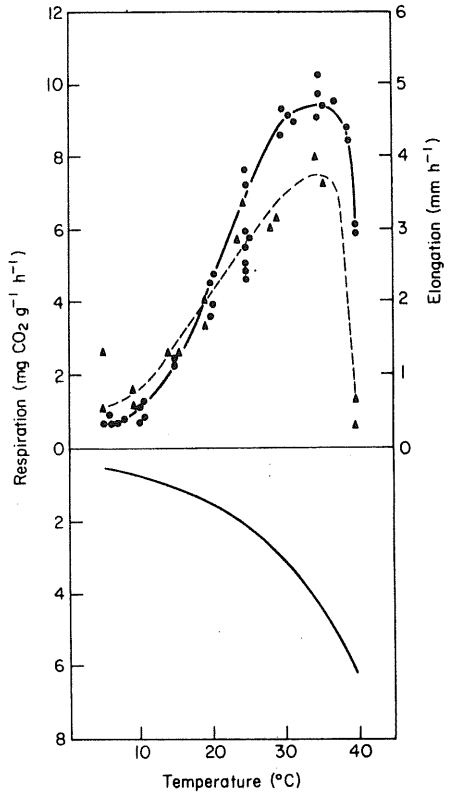

FIG. 4. Plant respiration and leaf elongation versus temperature in maize. For further explanation see Fig. 2.

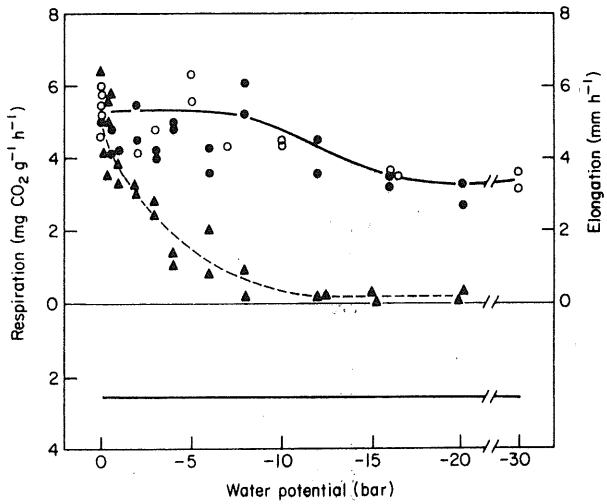

FIG. 5. Plant respiration and leaf elongation versus plant water potential in wheat. ●, indicate that the water potential was caused by PEG, ○, by mannitol. For further explanation see Fig. 2.

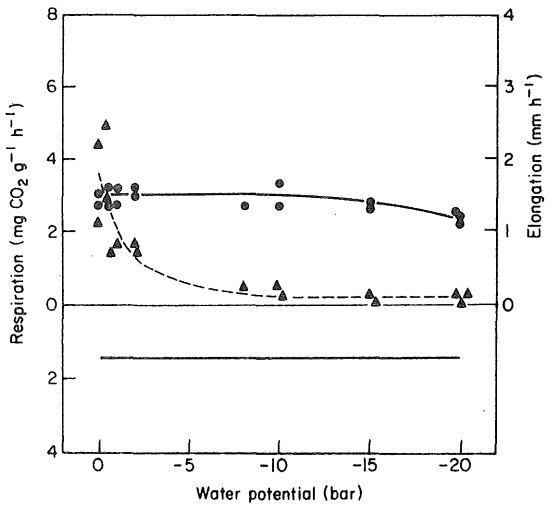

FIG. 6. Plant respiration and leaf elongation versus plant water potential in ryegrass. For further explanation see Fig. 5.

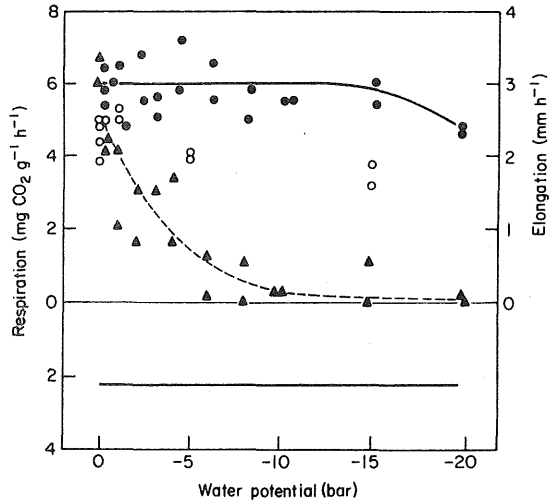

FIG. 7. Plant respiration and leaf elongation versus plant water potential in maize. For further explanation see Fig. 5.

Responses to water stress

Figures 5–7 present the responses of plant respiration and leaf elongation to water stresses from 0 to −20 or −30 bar. The rate of maintenance respiration is presented as in Figs 2–4, and it is supposed not to be changed by a briefly induced water stress.

The response of growth respiration rate to water stress is negligible in the three species until stresses of −8 to −15 bar, and is affected moderately at higher stresses. There are no significant differences between wheat, ryegrass and maize. There is a tendency of the

experiments performed with mannitol to have slightly lower respiration rates than the same experiment with PEG in maize, though not in wheat. It is not clear what causes these differences.

The lack of response of the rate of heterotrophic growth to water stress could be explained by compensation of a reduced shoot growth rate by a large stimulation of root growth. To investigate this possibility, root tips were clipped off at −15 bar. The rate of respiration of whole plants was not influenced. Hence, it is concluded that the rate of heterotrophic growth of the plant as a whole and of shoot and roots separately are not influenced directly by water stress.

Leaf elongation is strongly reduced at moderate stresses in the three species, but recovery occurs to a certain extent afterwards (e.g. Kleinendorst and Brouwer, 1970; Fig. 12).

The results of these experiments agree quite well with those of Boyer (1965, 1970) on leaves of field-grown cotton, maize, soya bean and sunflower.

Responses to the level of reserve carbohydrates

In wheat, ryegrass and maize plants, plant respiration increases with the RC level. In wheat a maximum rate of heterotrophic growth is attained at $5 \times 2{\cdot}13 = 10{\cdot}5$ mg SDM g^{-1} SDM h^{-1}, or 0·28 g g^{-1} day^{-1}, which is very high indeed. Ryegrass does not reach such high rates, while maize plants probably can exceed it at RC-levels beyond those obtained here. The observations of Ryle *et al.* (1976) that the growth respiration component of barley and maize decreases exponentially in darkness indicates also that the rate of heterotrophic growth depends strongly on the current RC-level. Jenner (1970), working with detached ears of wheat, found a response of starch synthesis to sucrose concentration in the endosperm similar to that of Fig. 8. Assuming that no growth occurs without RC present in the plant, the maintenance component of plant respiration used in this paper is derived by extrapolation to 0 mg TSC g^{-1} SDM of the lines fitted by eye through the measured respiration rates. In this way, maintenance respiration rates at 25 °C are 2·6, 1·5 and 2·2 mg CO_2 g^{-1} SDM h^{-1} for wheat, ryegrass and maize respectively. These rates are within the range reported in the literature, and it is assumed that these values can be applied over the whole range of RC-levels obtained. Consequences of these assumptions will be discussed below.

The scatter of observations in this experiment is larger than in previous ones. Apparently, the RC-level is not the only factor controlling growth and respiration in this group of experiments. The scatter in maize is particularly large, but one source of the scatter could be traced. Some maize plants used were much older than others, and weighed up to 8 g each. Grouping the data in plants weighing 0·5–2 g and those weighing 2–8 g, it appears that the smaller plants respond more strongly than old plants to similar RC-levels. From detailed observations by Gallagher and Lof (1971) on increase in leaf length, width and thickness, it was deduced that in similarly-raised maize plants of 1 g, about 0·7 g of the dry matter is in tissue that is still growing, while this fraction is only 0·4 in plants of 3·5 g. Expressing the growth rate per g SDM that is still growing (by multiplying the growth respiration of small plants by 1·5 and that of the bigger plants by 2·5) yields the curve of Fig. 11. Although there is still much scatter, it does give some support for the suggestion that it is more accurate to relate the growth rate to the amount of SDM still growing than to the total weight of SDM. Measurements made for another purpose (Penning de Vries, 1975*b*, Fig. 4a) show that small maize plants, exposed to 2 × higher light intensities for 24 h than those of Fig. 11 have respiration rates up to 12 mg CO_2 g SDM h^{-1}, and this observation fits well into Fig. 11.

Ryegrass and wheat could not be analysed similarly since the plants used were more uniform and a detailed leaf growth analysis, like that for maize, was not known to the authors.

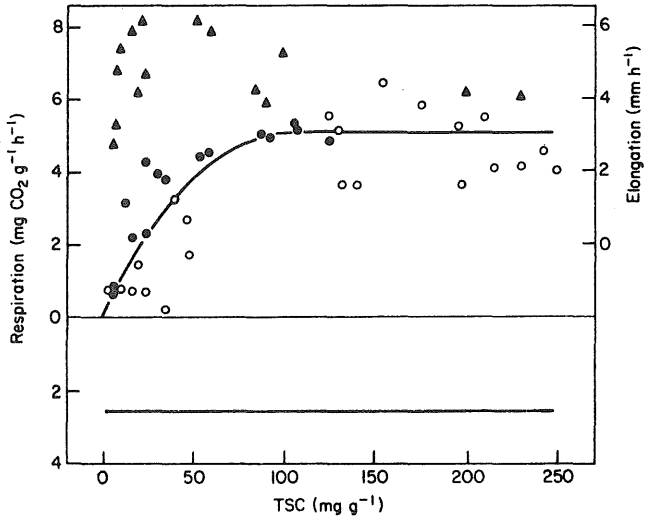

FIG. 8. Plant respiration and leaf elongation versus the level of reserve carbohydrates (mg TSC g^{-1} SDM). Closed dots represent points from a set of experiments in 1973, open dots indicate results from 1974. For further explanation see Fig. 2.

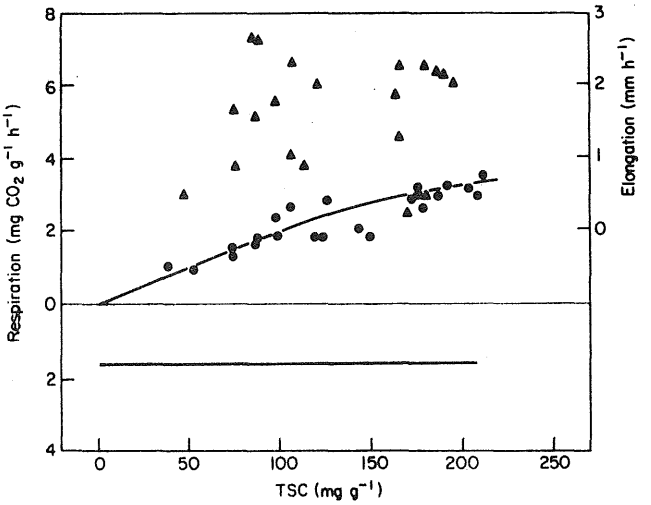

FIG. 9. Plant respiration and leaf elongation versus the level of reserve carbohydrates in ryegrass. For further explanation see Fig. 8.

The scatter in the rates of elongation is too large to indicate any response of these rates to the level of TSC.

Respiration during 24 h of darkness

The amount of substrate for growth and maintenance diminishes in darkness. With the knowledge of the relation between the RC-level and the growth rate, it should be possible to calculate the course of the RC-level, respiration and growth by using:

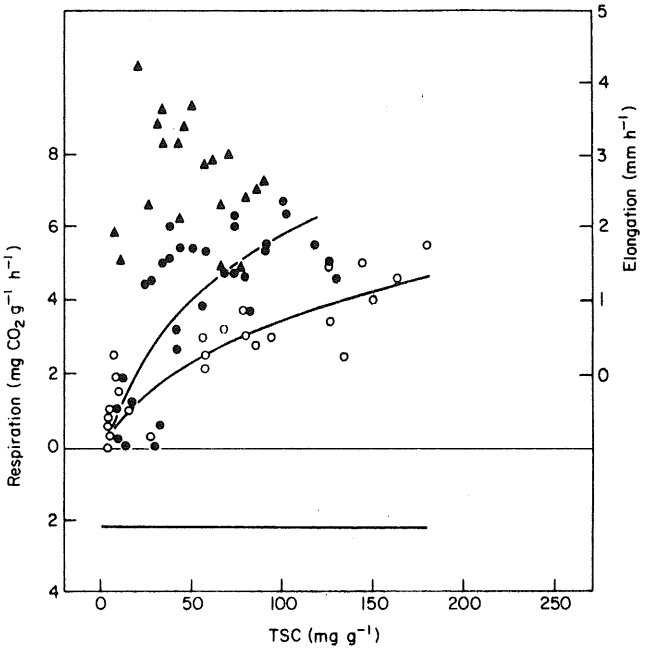

FIG. 10. Plant respiration and leaf elongation versus the level of reserve carbohydrates in maize. Observations with plants of 0·5–2 g are presented by ●, those of plants of 2–8 g by ○. For further explanation see Fig. 2.

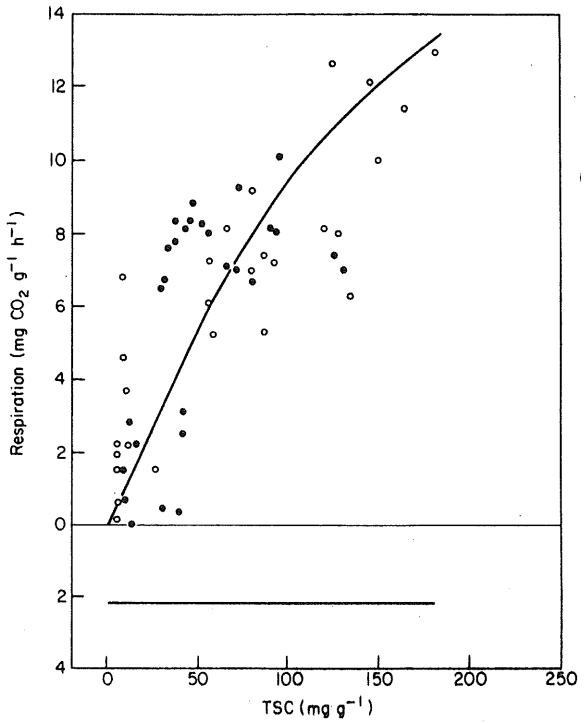

FIG. 11. Plant respiration (mg CO_2 g^{-1} growing structural dry matter h^{-1}) *vs.* the level of reserve carbohydrates in maize. For further explanation see Fig. 2 and text.

(a) eqn (2) that links SDM-growth, RC-use and respiration; (b) the ratio between maintenance respiration and RC-use (which is 1·63 g CO_2 g^{-1} RC) and (c) the initial RC-level. By calculating the course of the RC-level in time, it is possible to check the calculated carbon balance of the growing plant. Over a 24 h period, changes in the RC-level can be detected accurately, and the current respiration rates have only to be accumulated. Changes in the amount of SDM are still small and not easily determined with enough precision, but, even a partial balance is quite instructive.

The experiment and its simulation were performed first with ryegrass. For the calculations executed here; the simplifying assumption was made that all rates remain constant for 2 h, after which the RC-level is adjusted and new rates for the next 2 h are determined. Plants received the standard pretreatment, and hence had an initial RC-level of 190 ± 20 mg TSC g^{-1} SDM, and were measured at 25 °C without water stress. Figure 12 presents the observed and computed respiration rates during 24 h. Their trends are much alike, but the absolute levels differ somewhat. It is expected that the RC-level remained the major variable controlling the growth rate in this case. Since the initial RC-level was not established in this experiment, its actual value may have been below average: an initial RC-level of 160 mg TSC g^{-1} SDM would shift the calculated line almost exactly on top of the observed line.

The experiment was repeated at −20 bar. The real growth rate dropped steadily towards zero (Fig. 12). Supposing that the effect of the RC-level and water stress are independent, the course of the respiration was calculated by multiplying the effect of the RC-level on growth with the effect of water stress (0·8, Fig. 6). The simulated and real course of respiration differ significantly. This is interpreted as an increasing control of the growth rate by another factor than the RC-level. This factor causes a delayed effect of water stress on growth. This explanation resolves the conflict between the conclusion that water stress has little immediate effect on the growth rate (Figs 5–7) and the common experience that growth is reduced by water stress in experiments lasting a few days. If this interpretation is correct, it suggests that the reducing effect of the −20 bar water stress increased slowly from 0·8 immediately after onset of the experiment to 0·1 after 24 h. The RC-level of roots was always lower in the experiments described above than that of shoots. Only when exposed to water stress of −10 to −20 bar, did the RC-level of wheat and maize roots increase to some extent and in rygrass it almost doubled in a period of 1–1·5 h. If root cells and shoot cells respond similarly to a certain RC-level in their immediate environment, the generally-lower RC-level in roots causes them to grow more slowly than shoots. A process of translocation of RC from leaves to roots, stimulated by water stress provides a mechanism by which relatively more roots are formed in periods of stress. Such a mechanism may be an essential part of the functional balance between amounts of root and shoot, the existence of which was demonstrated earlier by Brouwer and De Wit (1968).

Unfortunately, the final RC-levels were not established in the 24 h experiments. The calculated use of RC amounts to 130 mg TSC g^{-1} SDM in the unstressed plants, and 112 mg TSC g^{-1} SDM in the stressed plants. Use of RC in similar experiments was 135 ± mg TSC g^{-1} SDM. These numbers do not contradict, although a better verification of the carbon balance is required.

The non-stress experiment was repeated twice with wheat. As a result of the standard pretreatment, the initial TSC-level must have been 125 ± 20 mg TSC g^{-1} SDM. Results of the experiment and its simulation are presented in Fig. 13. The calculated rate exceeds the observed rate considerably in the first 10 h of the experiment, but is smaller afterwards. The total consumption of RC calculated on the basis of Fig. 8 (130 mg TSC g^{-1} SDM) is not much different from the use of RC calculated on the basis of observed respiration rates (110 mg TSC g^{-1} SDM). The decrease in the RC-level in similar conditions was observed to be 110 ± 25 mg TSC g^{-1} SDM. It appears thus that the calculated

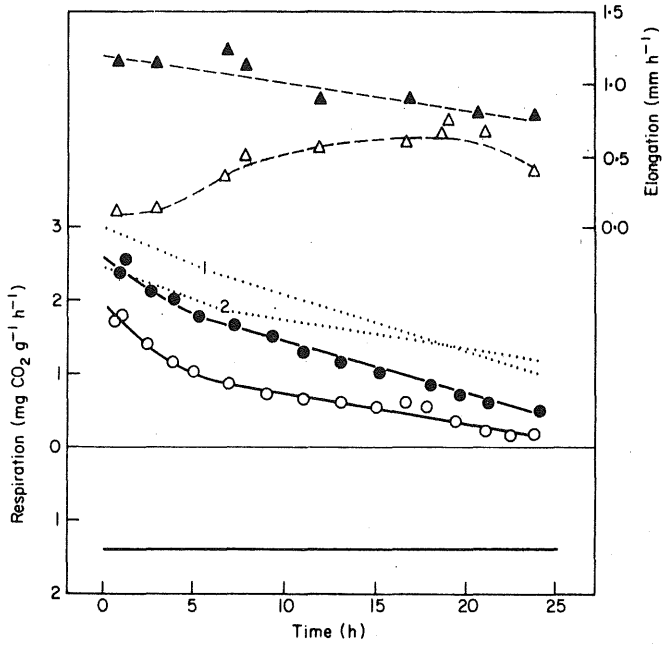

FIG. 12. Plant respiration (mg CO_2 g^{-1} structural dry matter h^{-1}) and leaf elongation (mm h^{-1}) of ryegrass during 24 h in darkness. Observed respiration rates of standard plants are presented by ●; the line labelled 1 gives the calculated course of the same plants. The respiration rates of plants at −20 bar are presented by ○; the line labelled 2 gives the calculated course in this case. The lower line of leaf elongation rates corresponds with the plant at −20 bar. For further explanation see Fig. 2 and text.

carbon balance may well be a realistic one, but also that the RC-level is not the only variable affecting the rate of heterotrophic growth in this case. Particularly between 2 and 12 h after the start of the experiment the actual rate of heterotrophic growth is lower than that expected on the basis of the RC-level, but it corresponds with the RC-level calculated from the observed respiration rates in the second part of the experiment. The cause of the reduction in growth rate in the beginning of the experiment is not known.

The respiration curve of Fig. 13 could be described by a double exponential decay function, but results of such an analysis should not be confused with those of Ryle *et al.* (1976). They analysed respiration data obtained over many days, and separated them into growth and maintenance components by mathematical analysis. Our measurements are taken over too short a time period to permit an analysis with a similar biological interpretation.

The rate of leaf elongation of ryegrass decreases slightly during the non-stress experiment. In the stressed condition, it is almost zero in the beginning but recovers temporarily to 0·7 of the unstressed rate. Thus the situation emerges that the leaf expands by water uptake, and with little protoplasmic growth to accompany it. It is quite obvious that the rate of leaf elongation is determined by different variables than is the rate of heterotrophic growth.

DISCUSSION

Although the application of the analysis of respiration data for the determination of the heterotrophic growth rate of whole, intact plants gives interesting results, it should not

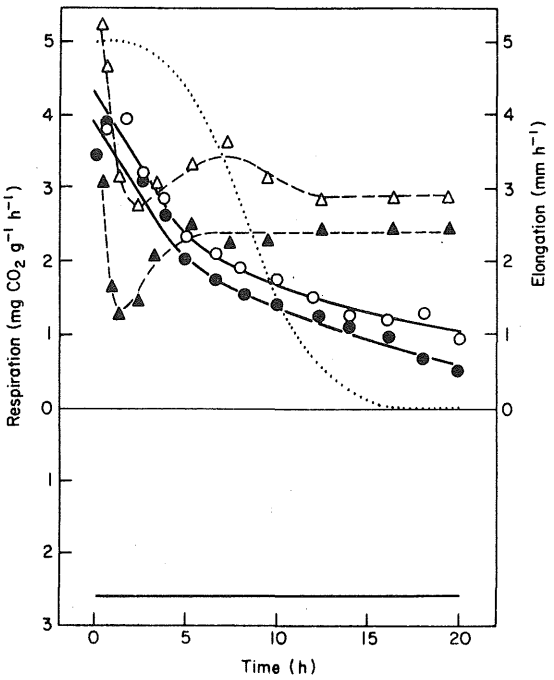

FIG. 13. Plant respiration and leaf elongation in wheat during 20 h in darkness. The dotted line indicates the course of the calculated respiration rate. The highest elongation rates were found in the experiment with the highest respiration rates. For further explanation see Fig. 2 and text.

be forgotten that a rigid test on the correctness of the approach has not yet been performed. That is: to measure the respiration during 24 h in darkness and to determine before and after this period the amount and composition of the SDM and the amount of RC. A complete carbon balance can then be constructed and checked

Such a test could reveal that one of the basic assumptions for the determination of the rate of maintenance respiration, namely that its value did not change as a result of the pretreatments to obtain different RC-levels, is not quite correct. It was suggested elsewhere (Penning de Vries, 1975*a*) that maintenance processes, protein turnover in particular, intensify with increase in total metabolic activity of the plant. However, adaptation of the rate of maintenance respiration to a new situation is not very fast. Ryle *et al.* (1976) suggested recently a half time for adaptation of about 40 h at 25 °C in similar experiments with maize and barley. If such rates apply also in the experiments described here, the rates of maintenance respiration shown in Figs 2–13 need modification: they should be 3·5, 2·1 and 3·1 mg CO_2 g^{-1} SDM h^{-1} for wheat, ryegrass and maize respectively for the water stress experiments. For the RC-level experiments, the original values are correct for 0 mg TSC g^{-1} SDM, but ought to be increased by 34 per cent at 125 mg TSC g^{-1} SDM and by 38 per cent at the highest TSC-levels obtained in wheat plants. For ryegrass and maize the corrections are 41 per cent at 190 and 65 mg TSC g^{-1} SDM respectively, and 54 per cent at the highest TSC-levels obtained. The curve representing the rate of maintenance respiration in the temperature experiments needs then to be moved by about 5 °C to lower values. Because of the procedure to calculate the rate of heterotrophic growth, such changes modify the form of the relations presented in the Figs 2–13. The consequences are not of an overriding importance, but

ought to be considered when this technique is applied or elaborated. Adaptation of the rate of maintenance respiration could be one source of the scatter of the data points of Figs 8–11.

There is need for clarification of the concept 'reserve material'. The plant does not consist only of short-living sugars and permanent structural dry matter, but of components with a range of degrees of permanence. Whether they remain in one form or are converted into the other depends sometimes on environmental conditions. The classification of different types of carbohydrates into permanent or temporary compounds is usually easy, although starch immediately takes a middle position. The situation is still more complex for proteins. Plants continue to grow heterotrophically in darkness, so that proteins or amino acids must have been stored in daytime since they do not reduce nitrate or make amino acids without light (in the experimental conditions used). This is reflected by eqn (2). Whether these are special storage proteins or not, is unknown to the authors. It seems that most proteins are broken down (and resynthesized) in the process of cell maintenance at one time or another, so that any distinction will probably be gradual. On a practical level, the complication arises that SDM and RC may have the same chemical form: cells may well need to maintain a minimal level of glucose to remain functional, which then, by definition, is part of the SDM. It will be analysed, however, as TSC, just like the carbohydrates that are really reserves. The same problem, but more intricate, exists for proteins, because the same protein molecule may function as an enzyme and be part of the SDM first, and serve as a source of amino acids later. For practical reasons, there may still be no better way to define 'reserve material' in plants than as those carbohydrates that yield easily to transport processes or metabolic conversions. This overestimates a little the amount of carbohydrates really available as reserves, and neglects completely that some proteins act as storage proteins.

When it is supposed that synthesis of SDM continues in darkness almost without change of its biochemical composition and without nitrate reduction taking place, eqn (2) becomes little modified in autotrophic organs, since they also are heterotrophic in darkness. If the composition of the SDM that is currently synthesized were to change in an important way when going from light to darkness (which seems unlikely but has not yet been tested) eqn (2) changes considerably, and this would have consequences for the interpretation of results presented in this paper. The assumption that the composition of the biomass synthesized does not change in an essential way can also be tested by a thorough check of the carbon balance.

As an improvement of the technique, it is planned to diminish considerably the time needed for a measurement of the rate of respiration of the plants in the enclosure. Already that rate can be derived very soon after plants are put in the enclosure and it is closed. The derivation is a mathematical analysis of the rate of change of the CO_2 concentration in the enclosure, which results from the flux of air entering the enclosure and its CO_2 concentration, and plant respiration.

The leaves of the experimental plants make up 0·8 or more of the total dry weight. Most of the whole plant respiration is thus from leaves. It appears then that leaf elongation responds in an almost similar way as leaf growth respiration to some factors, such as temperature but quite different to others, such as water stress. It reacts even the opposite way in some cases, as in ryegrass exposed for many hours to water stress. This demonstrates clearly that determining the rate of leaf elongation is unsuitable as a measurement of rate of increase of dry matter in leaves or in whole plants.

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