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Photosynthesis of Sugarbeets under N and P Stress: Field Measurements and Carbon Balance[1]

J. W. Cary[2]

ABSTRACT

Advances in crop management are limited by our inability to recognize changes in growth resulting from short-term fluctuations in plant environment. Simple, rapid, and nondestructive methods are needed to indicate daily and hourly rates of growth under field conditions. Because plant carbon balance is one possible approach to this problem, CO_2 gas exchange parameters were measured on *Beta vulgaris* leaves of field-grown plants to learn whether or not they might signal the onset of nitrogen and P stresses. The sugarbeets were grown on field plots of Portneuf silt loam (Xerollic Calciothid) soil and allowed to develop severe N and P stresses. Carbon dioxide exchange and stomatal resistance of intact plant leaves were measured with small leaf chambers during 1-min periods. Leaf water potentials were also measured in the field with a hydraulic press. The CO_2 compensation points, dark respiration, and osmotic pressures of excised leaf tissue were measured in the laboratory by standard methods. Photosynthesis per unit leaf area was reduced in advanced stages of N deficiency; however, none of the measurements satisfactorily indicated the onset of N or P stress because of heterogeneity between leaves. Calculations using the daily carbon balance of an average plant predicted the relative effects on root yield of 1) photosynthetic rate per unit leaf area, 2) photorespiration, 3) stomatal resistance to gas diffusion, 4) mesophyll resistance to carbon dioxide transfer, 5) size of the seedling tops in the spring, 6) partitioning of photosynthate between tops and roots near the end of the season, and 7) date of full plant cover. While all these factors are important, advances that can be made by manipulating most of them are limited. Increasing photosynthesis by reducing mesophyll resistance appears to offer the greatest potential for large yield increases.

Additional index words: **Dark respiration, CO_2 compensation value, Osmotic pressure, Stomatal resistance, Mesophyll resistance, Cold hardening, Water relations, Plant nutrition, *Beta vulgaris* yield.**

IN 1961, Musgrave and Moss described a portable field system for determining net assimilation and respiration of corn. They introduced their paper with the statement, "The summation of net assimilation over the season should be very nearly equal to total yield and should be related to the economic component of total yield. Therefore it seemed desirable to measure daytime assimilation and night respiration over short periods." The attractiveness of this proposal continues to lure investigators into similar studies, but significant results that can be used to solve practical problems continue to elude us. The failure of short term CO_2 exchange measurements to be a useful tool in plant breeding programs has been particularly disappointing (Moss, 1976).

Nevertheless, the growth of a plant must somehow be reflected through its carbon balance, i.e., the difference between photosynthesis and respiration. Photosynthesis may be described as:

$$P_N = \frac{C_a - \Gamma}{1.5\ r}\left(1 - \exp\ \frac{-r}{r_M}\right)$$

where P_N is the leaf CO_2 exchange rate or apparent photosynthesis, mg CO_2/(dm^2 hour); C_a the ambient air CO_2 concentration, ppm volume basis; Γ the CO_2 compensation point in light, ppm; r the leaf surface and air boundary layer CO_2 diffusion resistances, sec/cm; r_M characterizes the mesophyll sink strength for CO_2 which is equivalent to the more familiar but ambiguous "mesophyll resistance," sec/cm; and 1.5 a constant making the dimensions consistent (Cary, 1977). All of the variables in Eq. [1] can be measured except r_M which must be calculated. Respiration of aboveground plant parts can also be measured without great difficulty.

Loomis and Nevins (1970) have shown in the laboratory that N deficiency reduces P_N in sugarbeets. Terry and Ulrich (1973), also working under laboratory conditions with sugarbeets, showed that P deficiency reduced P_N and dark respiration, but in-

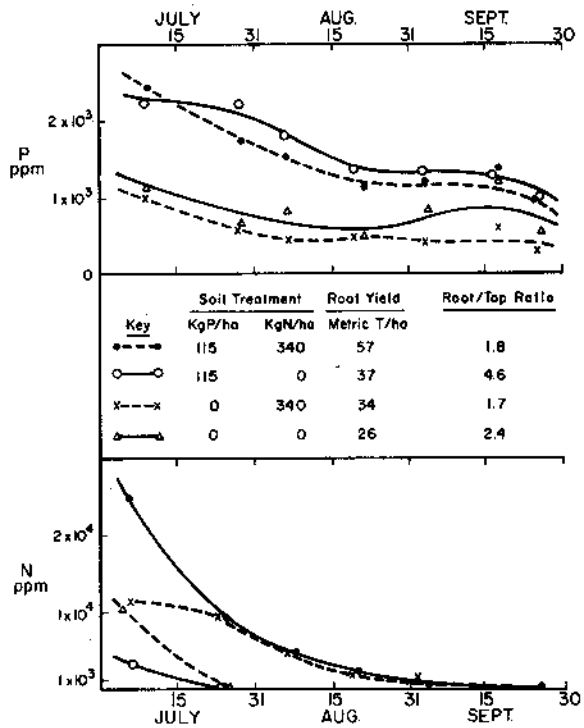

Fig. 1. The fresh root yields in metric tons/ha, root:top dry weight ratios, and levels of NO_3 and P in the sugarbeet petioles as affected by soil fertilization rates.

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[2] Soil scientist, Snake River Conservation Research Center, Kimberly, ID 83341.

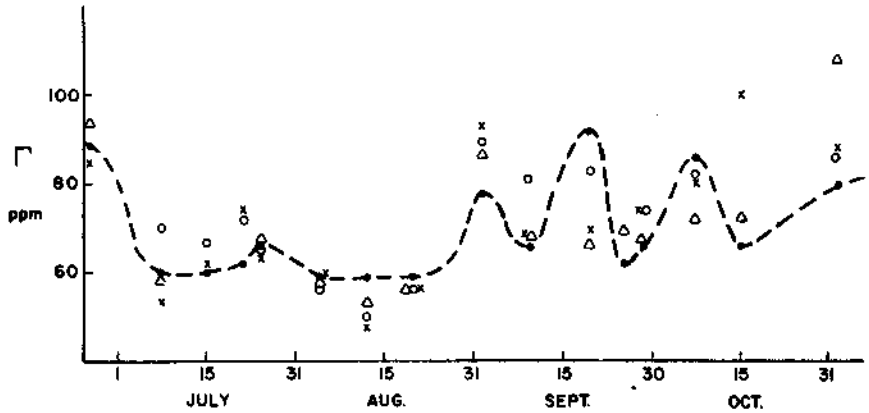

Fig. 2. Measurements of leaf CO_2 light compensation values. Most points represent an average value from two or three leaves. The symbols are defined in Fig. 1.

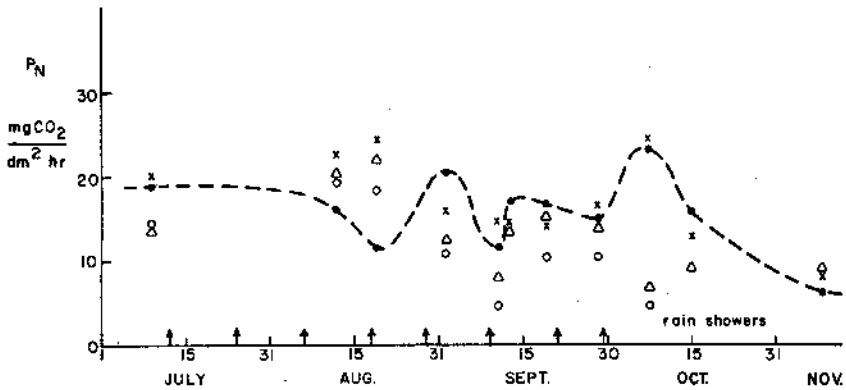

Fig. 3. Photosynthesis of sugarbeet leaves. Most points represent an average value for three leaves from randomly selected plants. Arrows indicate irrigation dates. The symbols are defined in Fig. 1.

creased Γ, r, and r_M. These results suggest that measurements of P_N, r, Γ, or dark respiration under field conditions of N and P stress might lead to practical methods for detecting early stages of nutrient stress.

METHODS

In April 1975, four plots each 15 m square were planted to sugarbeets. The soil was Portneuf silt loam (Xerollic Calciothid) that had been leveled a number of years before and was low in N and P. The plots were fertilized at the rates shown in Fig. 1 and were periodically irrigated from furrows just before the soil water fell to a matric potential of —1 bar at the 40-cm depth.

Values of P_N, r, and Γ were measured during the midday throughout the growing season, as described previously (Cary, 1977) with one exception occurring between 1 June and 14 July when Γ was measured in the field rather than in the laboratory. This was done under full sunlight in a plastic chamber using ice and evaporation for cooling which did not provide precise temperature control. Dark respiration was measured in the laboratory by placing 5 g samples of fresh leaves in a black jar submerged in a constant temperature bath, and passing CO_2-free air over the sample into an infrared analyzer at 500 ml/min. Osmotic potentials were measured with a psychrometer in the laboratory and leaf water potentials with a hydraulic press in the field during mid-day.[3]

[3] Campbell Scientific, Inc., Logan, Utah. Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by USDA.

RESULTS

Nutrition levels, root yields, and root:top ratios of the plants on the plots are shown in Fig. 1. Plants on the plots that received no N or no P developed lower petiole concentrations than one ever now encounters in commercial fields, and deficiency symptoms were clearly visible in June. The plants on the control plot had adequate N and P and showed no signs of nutrient stress.

Measurements of dark respiration of leaf tissue made intermittently throughout the season did not show any consistent difference between the control and N or P stressed plots. Values ranged from 2 to 8 mg CO_2/(dm² hour) at 25 C. The highest values came from young expanding leaves during the first hour of darkness. The dark respiraiton was temperature-sensitive during the first hour of darkness, increasing by 50% or more when the temperature was raised from 20 to 30 C. However, after 8 hours or so in the dark, all respiration rates fell to near 3 mg/(dm² hour).

Measurements of Γ made at random on leaves of intermediate age throughout the season are shown in Fig. 2. There appears to be no trend related to N or P levels. The season averages for the four treatments only varied from 71 to 73 ppm CO_2. In general, the values were lowest in July and August. The higher trend in June may reflect the method of measurement (see Methods section), as well as the sampling

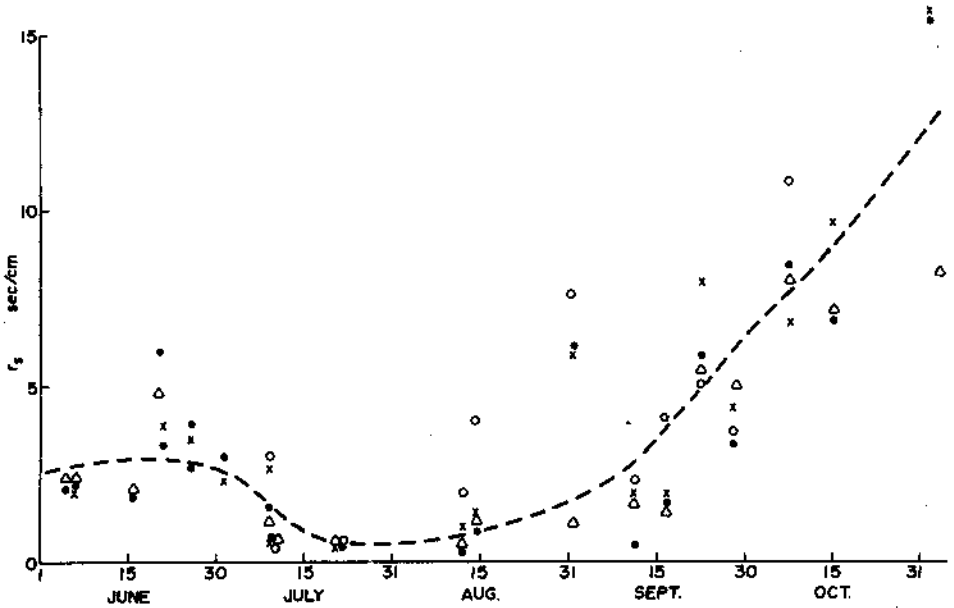

Fig. 4. Seasonal changes in stomatal plus cuticular resistance to CO_2 diffusion. Most points represent an average of at least three observations from randomly selected leaves on different plants. The dashed line approximately follows the control treatment. The symbols are defined in Fig. 1.

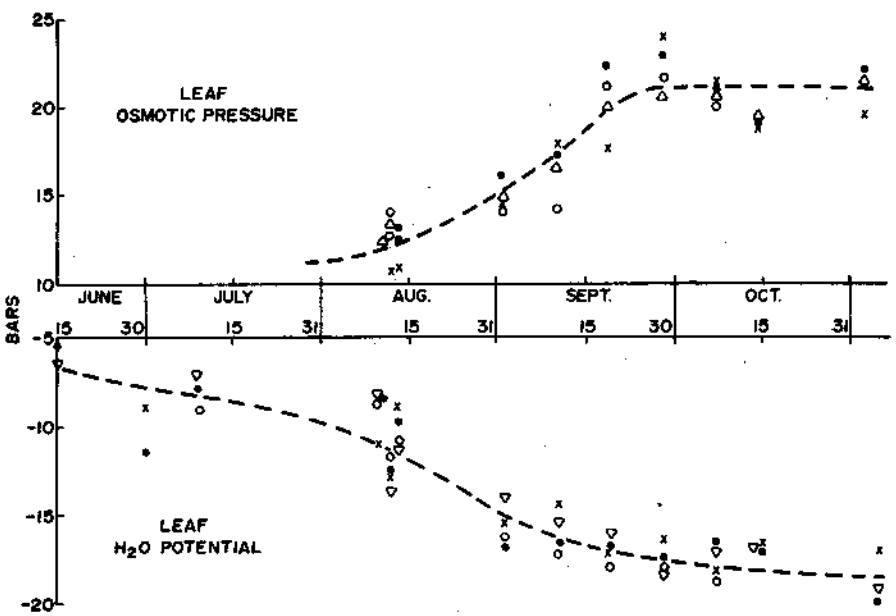

Fig. 5. Leaf osmotic pressures and water potentials from randomly selected plants. The symbols are defined in Fig. 1 and most points represent the average of two leaves on different plants.

of generally younger leaves which have higher Γ values.

Values of P_N measured throughout the season are shown in Fig. 3. The individual measurements from which these points are averaged showed some variations even larger than those of ± 15% reported by Naylor and Teare (1975) who sampled several species of plant leaves using a $^{14}CO_2$ method for field measurements of P_N. Most of the variation was due to real differences between individual leaves on the same treatment because measurements of P_N made on the same leaf over consecutive 1-min periods generally agreed within ± 2 mg CO_2/(dm² hour). In spite of the scatter of points, a treatment trend is indicated in Fig. 3 by the generally lower values for the N-deficient plants as the season progressed. The seasonal average value of P_N for the low N, high P plants was only 12 mg CO_2/(dm² hour). The low N, low P was 14 while the control was 17 and the high N, low P treatment was 19 mg CO_2/(dm² hour).

Figure 4 shows mean values of leaf surface resistance. There was no obvious separation of points due to the fertility treatments.

Average values of leaf water and osmotic potential are shown in Fig. 5. Again there was no indication of a separation of data points due to fertility treatments. Symptoms of cold-hardening, such as increased osmotic pressure and decreased stomatal activity, coincided in

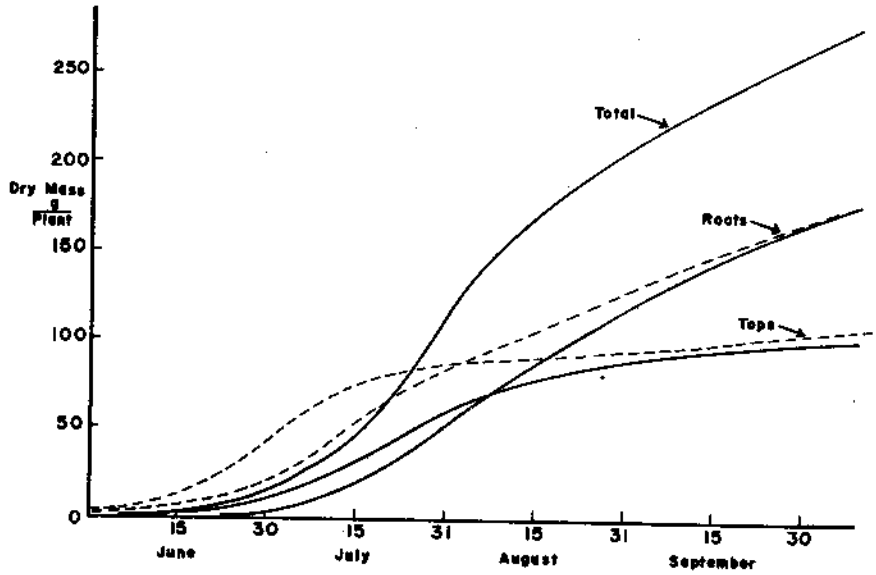

Fig. 6. The calculated plant dry mass accumulation of the average plant in the control treatment is shown by the solid lines. Dashed curves represent data reported by Follett et al. (1970).

September with shorter days and some night temperatures near freezing. It is interesting that Γ values were higher during this period than during mid-summer.

DISCUSSION

Even though there were large differences in yields, the treatments did not generally produce obvious trends in measured values of CO_2 exchange rates per unit leaf area, stomatal resistance, CO_2 light compensation points, osmotic pressure, or leaf water potentials. If trends between treatments existed, they were largely masked by the heterogeneity of the data. The obvious question is, then, how large a difference in P_N, r, or Γ must develop in order to cause a significant change in root yield, This question can be answered to a first approximation with a simple carbon balance calculation.

Carbon Balance

The total dry weight of any plant is closely related to the sum of its daily carbon fluxes. One way to describe such a system is to consider the carbon intake through the leaves as a daily "interest" rate, with growth analagous to rate of monetary capital gain in a savings account. This approach was possibly first proposed by Blackman (Rickman et al., 1975).

The early season leaf weights are then given by the interest formula compounded daily

$$m_{k+n} = m_k (1 + i)^n \qquad [2]$$

where m_k is the dry mass of the leaves at the beginning of n days, and i the average daily interest per unit leaf area expressed in decimal form. When full leaf ground cover is reached, leaf shading and senescence increases as fast as new leaf growth, so that the interest no longer compounds daily but dry leaf mass accumulates as:

$$\Delta m = m_c n i \qquad [3]$$

where m_c is the leaf mass at full cover. Of course, i changes throughout the growing season, so in practice equation 2 is used to calculate leaf mass increases over time periods containing n number of days in which i may be considered constant, beginning with a known plant top weight early in the season. As soon as the plant's leaf mass is great enough to provide full cover, Eq. [3] is summed over a number of short periods of constant i to arrive at the leaf mass at the end of the season.

The daily interest rate values used in Eq. [2] and [3] depend on the carbon balance of the leaves according to the relation

$$i = 0.67\,\gamma\left[\frac{\overline{P}_N\, t_L\, M - t_D\, R_D}{\overline{m}}\right] \qquad [4]$$

where t_L and t_D are, respectively, the hours per day of average photosynthetic rate, P_N, and of average dark respiration, R_D; M is the daily fraction of fixed carbon that remains in the tops; m the average mg of dry matter per dm^2 of leaf; 0.67 the conversion factor between CO_2 and dry matter; and γ the fraction of plant top composed of leaf blades.

The growth of roots is the sum of daily increments of fixed carbon leaving the top, minus the respiration loss, or

$$W_{k+n} = A[W_k + 0.67\, n\, (1 - N)\, \overline{P}_N\, (1 - M)\, \frac{\overline{W}}{\overline{m}}\, t_L] \qquad [5]$$

where W_k and W_{k+n} are the root's dry mass at the start and end of n days, and $\overline{W}$ is the average dry mass of leaf blades that export carbon during the period of n days. The maximum value of $\overline{W}/\overline{m}$ is taken to be that at full ground cover. The term $\overline{P}_N$ is again the average daily rate of photosynthesis per unit leaf area expressed as mg CO_2/(dm^2 hour), N is the fraction of translocated photosynthate from the tops that is lost daily by root respiration, and A is a fraction that accounts for fine roots lost at harvest and the maintenance respiration rate of the bulk root (McCree, 1976).

Table 1. Values used to calculate yields. The symbols are defined by equations 2 and 4. The values of i are calculated from equation 4 and apply to the control treatment with $P_N = 17$.

Time period	t_L	t_D	$\bar{m}$	M†	i
8 to 25 June	13.0	8.5	480	0.78	0.161
26 June to 15 July	13.0	8.5	580†	0.65	0.107
16 to 31 July	12.5	9.0	580†	0.45	0.062
1 to 31 August	11.8	10.2	660	0.35	0.031
1 September to 8 October	10.0	11.8	750	0.30	0.011

† Estimated values.

The carbon balance calculation given by Eq. [2] through [5] was used to show in Fig. 6 the growth of an average plant on the control plot. Input numbers are shown in Table 1. The average $\bar{P}_N$ value of 17 mg CO_2/ (dm^2 hour) given by the points in Fig. 3 was used for $\bar{P}_N$ over the whole growing season. The calculation was begun on 8 June when the average plant dry top mass was known to be 0.25 g and ended on 11 October when the average plant total dry mass was known to be 98 g of top and 180 g of root. Other values taken as constant were $\gamma = 0.8$, $N = 0.2$, $A = 0.9$, and $R_D = 3$. The growing season was divided into five parts, taking each with the average values of i shown in Table 1. Evapotranspiration measurements indicated full cover on 15 July (Dr. J. L. Wright, unpublished data, personal communication). Values of M were chosen so that the predicted yield of tops and roots would correspond with those observed at the end of the season with a 15 July full cover leaf area index of just over 3. Measurements of top:root ratio values throughout the season indicate the changing values of M and those used here do agree with data reported by Follette et al. (1970). Fick et al. (1973) have developed a detailed computer simulation that can give, among many other things, values of M for sugarbeets. Their model, subgro, is much more complex than the carbon balance calculation used here. Nevertheless, Eq: [2] through [5] are fundamental and inclusive, besides being simple enough to use with a hand-held calculator. The calculations can be made as detailed as the accuracy of the input data justify.

The dashed curves shown in Fig. 6 represent the top and root growth of a plant from a control plot in the study reported by Follett et al. (1970). Their work was done in a similar climate and the yield of their control plot was almost identical to the yield of the control in this study. Consequently, their root production at the end of the season could be matched with a factor to the average root mass produced here, and their cumulative growth curves transformed to the scale used in Fig. 6. The root:top ratio they reported was a little smaller at the end of the season, and their average plant top size was larger on 8 June than those of the control plot reported here. Nevertheless, their observed growth curves had shapes similar to those calculated for this case, particularly in the crossover characteristic for root:top mass gains. If the growth curves represented by the solid lines had been developed with a larger value for $\bar{P}_N$ earlier in the season and a smaller value later in the season, they would have approached the shapes reported by Follett et al. (1970) even more closely.

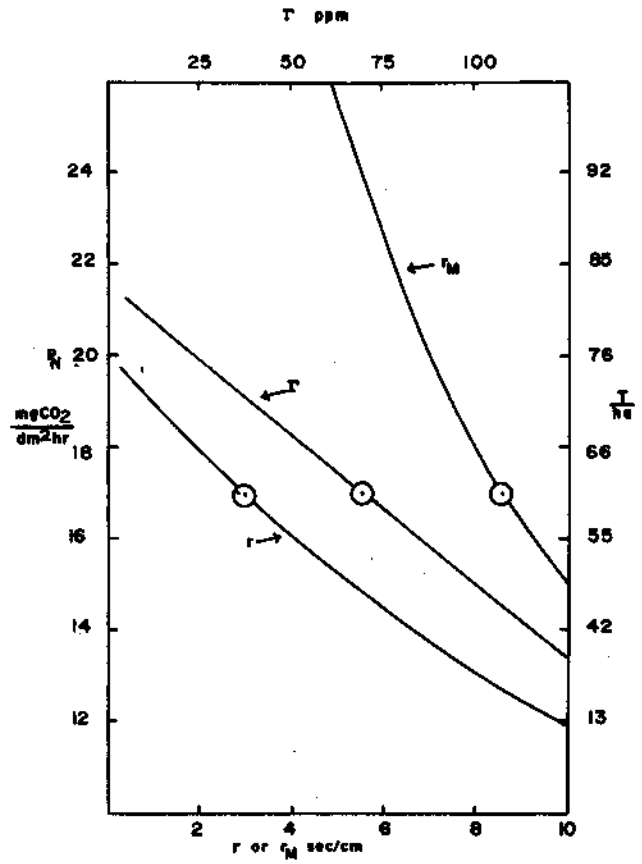

Fig. 7. Carbon balance calculations of the fresh root yields in metric tons/ha as a function of the growing season's average daily photosynthetic rate. The three curves show the effect of changes in stomatal resistance, mesophyll resistance, and CO_2 compensation points on both photosynthesis and root yield. Each curve is formed with the normal values for the other two curves as constants in equation 1. Normal values are shown by the circles that are, in fact, averages of the data in Figs. 2, 3, and 4 for the control treatment.

Implications of the Carbon Balance Calculations

The calculations are useful for demonstrating the relative importance of factors that control sugarbeet yield. For example, if the seedling tops on the control plots had reached dry weights of 1.25 g rather than 0.25 g on 8 June, root yield would have been increased by 10%. In this study, the seedling top mass of the P-deficient treatments was only 0.03 g on 8 June, so even if the P deficiency had been corrected at that time the yield would still have been reduced. Hastening the date of full cover is also important, since each week gained can increase yield by 5% or more. If one could control growth with hormone treatments so that top growth stopped and all dry matter accumulation after 10 August went into root storage, yields could be increased by as much as 20%.

Most striking, however, is the very sensitive relation between the season's average photosynthetic rate and root yield which may be seen by comparing the two ordinates in Fig. 7. Equations [2] to [5], with

the values given in Table 1, were used to find this functional relationship. The average values of r, Γ, and P_N given for the control plots in Fig. 2, 3, and 4, were also used with Eq. [1] to show the relation between root yield and r, Γ, and r_M. For example, if the seasonal average value of r could be reduced from 3 to 1 sec/cm, Fig. 7 shows the yield would rise from 59 to 71 metric tons/ha. If the photorespiration of the leaves could be reduced so that the CO_2 compensation point fell from 70 to 15 ppm, the yield would rise from 59 to 78 metric tons/ha. However, by far the greatest potential for increasing yield lies in decreasing r_M, i.e., in reducing the resistance to transport of CO_2 in the mesophyll and/or the CO_2 concentration around the chloroplasts.

Figure 7 clearly illustrates the difficulty in using field measurements of photosynthesis, stomatal resistance, leaf water potential, or CO_2 compensation points to detect the onset of stress conditions before yield is significantly reduced. Changes in yield of 6 metric tons/ha (10%) may be caused by changes in seasonal average values of only 1 sec/cm in r, 15 ppm in Γ, or 1 mg CO_2/(dm^2 hour) in $\overline{P}_N$. These small values compared to the heterogeneity shown by the data in Fig. 2 to 4 indicate the challenges ahead. Indeed, Moss (1976), in discussing the problem of selecting genotypes for CO_2 fixation capacity, states, in part, that, "there is great variability for measurements within a genotype at a given time and many replicates are required to get reliable rankings . . . research is needed on methodology to measure photosynthetic rates . . ."

No claim is made that the relations shown in Fig. 7 between $\overline{P}_N$ and yield are of unquestionable accuracy at either the high or low extremes. The uncertainty arises primarily from the lack of information on root respiration and factors such as nitrogen nutrition that control the partitioning of photosynthate between tops and roots under field conditions. Both of these quantities were assumed to be independent of the values used for $\overline{P}_N$ in Fig. 7. Nevertheless, the method is fundamental and the accuracy can be improved as better data become available. In the interim, the relations shown in Fig. 7 illustrate the problems we face, and suggest directions of most fruitful research.

Though the variation between measurements of P_N on individual leaves is frustrating, the occasional high values do indicate that greater growth potential exists but is suppressed by environmental or physiological factors that we do not yet appreciate. The variation in P_N among leaves on the same treatment is largely related to changes in the parameter for mesophyll sink strength for CO_2, i.e., r_M. Large r_M values may be due to changes in physical resistance to CO_2 transfer between the internal stomatal cavities and the chloroplasts, or they may be due to high concentrations of CO_2 near the chloroplasts resulting from either membrane permeability or carboxylation problems.

CONCLUSIONS

1. Even though photosynthesis per unit leaf area, CO_2 compensation points, stomatal resistance, leaf water potential, and dark respiration are known to change during periods of N and P stress in controlled laboratory studies, the changes under field conditions are marked by heterogeneity and sampling problems such that none of these measurements yet provide a practical basis for determining the onset of mild stress conditions in sugarbeets.

2. The simple carbon balance calculation shows that a change of 6% in the seasonal average photosynthetic rate measured on a per-unit leaf area basis can result in a 10% change in root yield. Until confidence in the accuracy of characterizing photosynthetic rates under field conditions can be significantly increased, the utility of carbon balance method will be of limited value as a practical management tool for sugarbeet production.

3. Management errors made early in the season will generally be more costly than those made later because of the importance of reaching full ground cover at an early date. Developing a uniform stand, promoting vigorous early growth, eliminating disease, and controlling the partitioning of photosynthate between tops and roots may each affect yield 10 or 20%, but the key to largest increases in production lies in increasing photosynthesis per unit area of leaf surface.

4. In the case of sugarbeets, reducing either the stomatal resistance to gas diffusion or the amount of photorespiration are limited to increasing the average rates of photosynthesis by less than 25%. However, if the leaf mesophyll sink strength for CO_2 could be increased only moderately, photosynthesis and production could rise dramatically.

ACKNOWLEDGMENTS

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