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Methods of Growth Analysis in Field-Grown Soya Beans (*Glycine max* (*L.*) Merrill)[1]

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ABSTRACT

Investigations on the growth analysis of soya beans (*Glycine max* (L.) Merrill) involved measurement of shoot and leaf growth by (a), classical equations, and (b) regression procedures. The experiments were carried out at the Western Iowa Experimental Farm, Castana, on Ida Silt loam soil during 1975 and 1976. Soil-water status in the experimental plots was altered by covering the inter-row strips with 4-mm black plastic film and by irrigation in 1976. Relative Growth Rate and Relative Leaf Growth Rate declined with age of the plant because the level of self-shading increased. Net Assimilation Rate and Crop Growth Rate, calculated by using the regression procedure for the uncovered plots, showed a marked increase in the latter part of the growing season due to a combination of decreasing leaf area and increasing dry weights. Classical methods of analysis fail to show this feature because of the non-linear relationship between leaf area and dry weight. It is proposed that in the future more attention should be paid to the use of the appropriate mathematical function describing the changes of leaf area and dry weight with time. Analysis of statistical parameters showed the superiority of using regression procedures.

INTRODUCTION

Techniques used to quantify the components of growth are collectively known as 'growth analysis'. Such procedures represent the first step in the analysis of primary production and are the most practical methods of assessing net photosynthetic production (Iwaki, Monsi and Midorikawa, 1966).

An important requisite for analyzing plant growth is the availability of accurate data regarding the dimensions of the assimilatory apparatus, such as leaf area and the resultant biomass accumulation expressed as the dry weight of whole plants or parts. These data should be obtained at regular intervals during the crop growing season. From these data, it is possible to calculate various growth indices. The indices commonly in use are: Relative Growth Rate (RGR), Relative Leaf Growth Rate (RLGR), Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Crop Growth Rate (CGR), Leaf Area Index (LAI), and Leaf Area Duration (LAD).

Watson (1952) reviewed the classical techniques employed in growth analysis. Mean values of growth indices may be calculated by using the formulae:

$$\mathrm{RGR} = \frac{(\ln w_2 - \ln w_1)}{t_2 - t_1} \tag{1}$$

$$\mathrm{NAR} = \frac{(w_2 - w_1)(\ln A_2 - \ln A_1)}{(A_2 - A_1)(t_2 - t_1)} \tag{2}$$

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$$\text{RLGR} = \frac{(\ln A_2 - \ln A_1)}{(t_2 - t_1)} \tag{3}$$

$$\text{CGR} = \frac{(W_2 - W_1)}{(t_2 - t_1)} \tag{4}$$

where w_2 and w_1 are the dry weights, A_2 and A_1 are the leaf areas, W_2 and W_1 are plant weights per unit area of ground at times t_2 and t_1, respectively.

Briggs, Kidd and West (1920) in their proof of eqn (2) assumed that changes in plant weight and leaf area were proceeding exponentially. Many years later, Williams (1946) pointed out that, there are limitations in the use of these equations and that the calculation is feasible only if A and W are linearly related. Radford (1967) concluded that much confusion resulted from the inappropriate use of growth analysis formulae and from a misunderstanding of the assumptions involved in their derivation. Also, the time interval calculation of growth indices is valid only if the sampling errors of the primary values of W and A are considerably smaller than the changes in these values from the effects of the factors being investigated (Kvet, Ondok, Necas and Jarvis, 1971).

A new concept of growth analysis was evolved that uses regression procedures, of which Kvet *et al.* (1971) provide a complete description: The principle consists of the choice of a suitable mathematical function, represented by a smooth curve, which is fitted to the recorded values of W or A so that it approximates the real growth curve.

Vernon and Allison (1963) used parabolic functions of both W and A.

$$W = a + bt + ct^2 \tag{5}$$

$$A = a' + b't + c't^2 \tag{6}$$

where a, b, c and a', b', c' are equation parameters to be determined.

Hughes and Freeman (1967) used exponential functions

$$W = \exp\,(a + bt + ct^2 + dt^3) \tag{7}$$

$$a = \exp\,(e + ft + gt^2 + ht^3) \tag{8}$$

where a to h are equation parameters. In general, the cubic expression does not contribute significantly to the accuracy of the fit.

The growth indices are then calculated, and their time courses are constructed from values given by the fitted curves. Calculation of growth indices from the fitted curves is a relatively new approach, and, in many situations, may be sounder than employment of the classical formulae. Radford (1967) presented the basic formulae, together with necessary and sufficient conditions for their use, but provided few experimental data. The present investigation is concerned with a detailed analysis of the growth of soya beans under field conditions for which extensive experimental data were collected. Evaluation is made of the two methods of calculation of growth analysis, namely the classical and regression procedures.

MATERIALS AND METHODS

The experiment was conducted in Ida silt loam soil [fine, silty, mixed (calcareous) mesic family of Typic Udorthents] at the Western Iowa Experimental farm, Castana, Iowa. The soil-water status was altered by covering some of the inter-row strips of soil in the plots with 4-mm black plastic film. The purpose of this was to prevent rain from seeping into the ground and to reduce evaporation losses of soil water.

The plots were arranged in a randomized block design with four replications. Each replicate consisted of two plots, one plot covered with plastic film and the other left uncovered. Individual plots were 50 m long and seven rows (1 m apart) wide. Inoculated seeds of 'Wayne' soya beans (*Glycine max* (L.) Merrill) were planted on 12 May in 1975 and 1976. The uncovered plots were irrigated twice during the growing season in 1976 to provide a range of moisture stress conditions. The treatments will be referred to as 'covered' and 'uncovered' to identify the black plastic film-covered and bare plots, respectively.

In both years, whole plants harvested at ground level were sampled weekly beginning at 35 to 40 days after planting. On each sampling date 10 plants were selected randomly from each replicate block. Each plant was separated into leaves, stems, petioles, pods and seeds. Leaf area was determined with a portable leaf area meter. Plant parts were dried to constant weight in a forced-draft oven at 65 °C and then weighed. Samples were obtained on 13 and 12 different dates in 1975 and 1976, respectively.

The classical methods of growth analysis were tested by calculating RGR, NAR, RLGR and CGR from eqns (1) to (4). The regression procedure of growth analysis was tested by fitting curves through the recorded values of W and A, as described by the mathematical functions in eqns (7) and (8). The cubic term was eliminated from these equations because it was not significant. The growth indices were calculated by using the equations presented by Buttery (1969).

RESULTS AND DISCUSSIONS

Basic to the calculation of growth indices is an understanding of the time relationship between leaf area and dry weight. The choice of mathematical functions that can adequately describe this relationship is critical in the regression procedures of growth analysis. Figures 1 and 2 show the changes in leaf area and dry weight with time. Use of eqns (7) and (8), eliminating the cubic term, showed a very good fit, with R^2 values greater than 0·99 in both cases.

Important in the calculation of NAR by classical methods is the assumption that dry weight is linearly related to leaf area. Figures 3 and 4 show the relationship between leaf area and d. wt in 1975 and 1976, respectively. It is evident that linearity between the two measures is true only for a short period. During 1975, the two treatments showed a close relationship. But, in 1976, low values of leaf area and d. wt were observed for the covered plots, probably because of moisture stress.

Buttery and Buzzel (1974) stated that the quadratic equations that they used to relate ln leaf area and ln dry weight to time had a builtin bias, which became obvious when comparing the residuals (observed values—values calculated from the regression equations) of the two populations they examined. Positive residuals in one of their populations tended

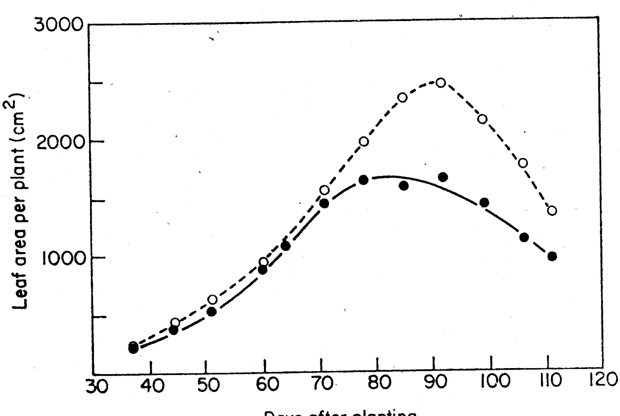

FIG. 1. Changes in leaf area with time for the covered (●—●) and uncovered (o---o) plots in 1976.

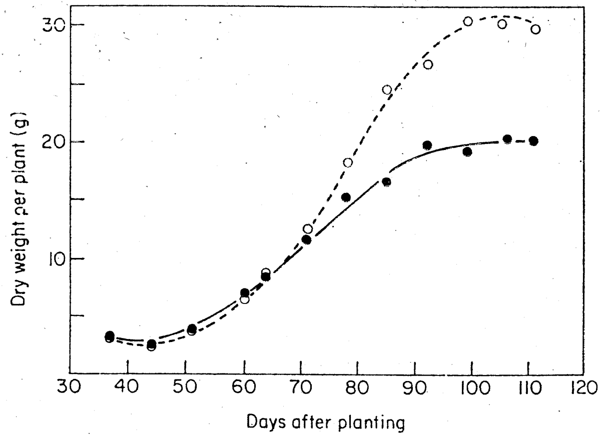

FIG. 2. Changes in dry weight with time for the covered (●—●) and uncovered (o---o) plots in 1976.

to be associated with negative residuals in the other population at any given time. Hence, the effect of population on NAR was exaggerated by the regression technique, especially early in the season. One of the reasons for this discrepancy was the small number of samples used in their investigation.

The following discussion of growth indices is largely limited to the data obtained in 1976. In our study, the regression equations fitted were based on 13 samples during 1975 and 12 samples during 1976. Each data point was the mean of 40 plants so that the sampling errors were greatly reduced. Figure 5 shows a plot of the residuals for the regression fit for ln leaf area and ln d. wt. The residuals show a random distribution with a fairly good correlation between the covered and uncovered plots. It is evident in this case that regression equations could be used without introducing any bias.

Figure 6 represents the changes in Relative Growth Rate (RGR) during the growing season based on both the classical and regression procedures. It is evident that, in general, the uncovered plots showed a greater relative growth rate than the covered plots, probably because of a better plant-water status. The regression procedure removes the short-term

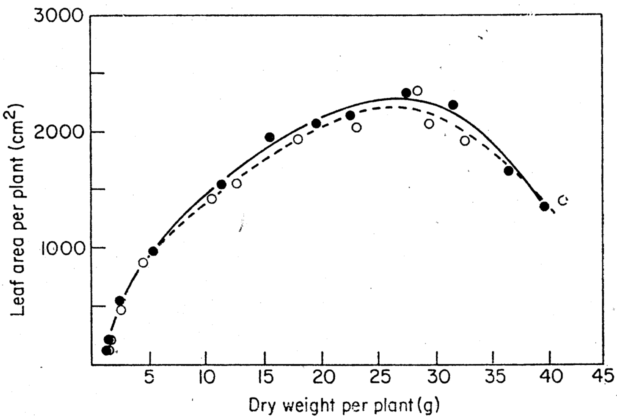

FIG. 3. Relationship between leaf area and dry weight for two treatments during 1975. (●—● covered, o---o uncovered.)

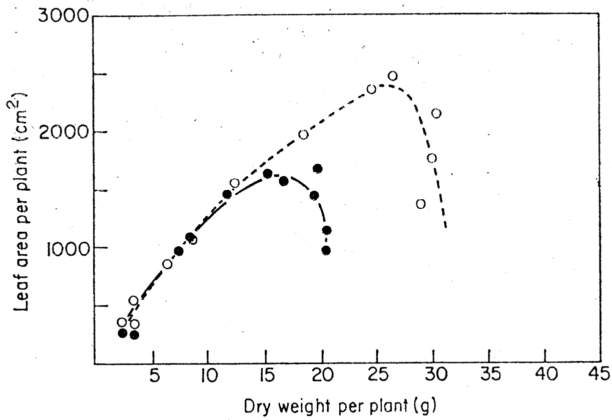

FIG. 4. Relationship between leaf area and dry weight for two treatments during 1976. (●—● covered, o---o uncovered.)

variations not eliminated in the classical methods, which makes it easier to follow the course of plant productivity.

Relative Leaf Growth Rate as a function of time is shown in Fig. 7. The patterns of the curves are similar to those observed in Fig. 6. Much of the decline in RGR and RLGR can

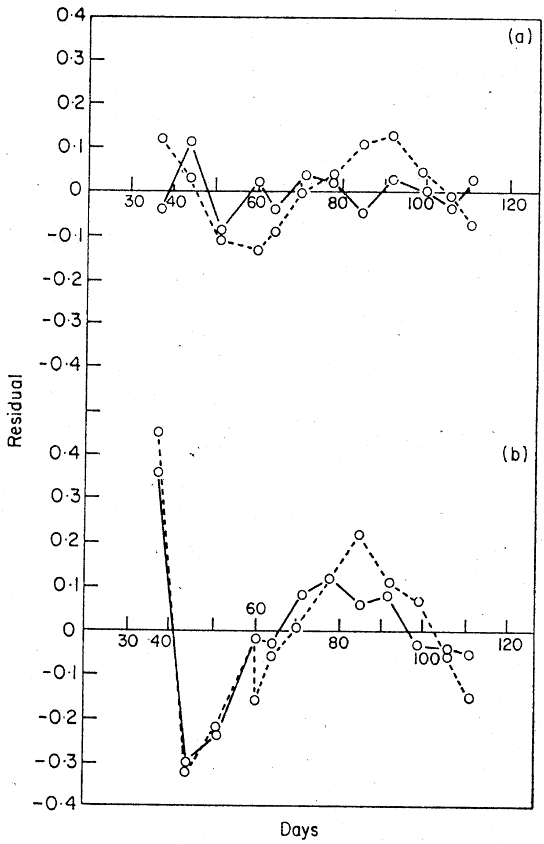

FIG. 5. Effect of two treatments with time on the residuals for (a) ln leaf area, and (b) ln d. wt (o—o covered, o---o uncovered).

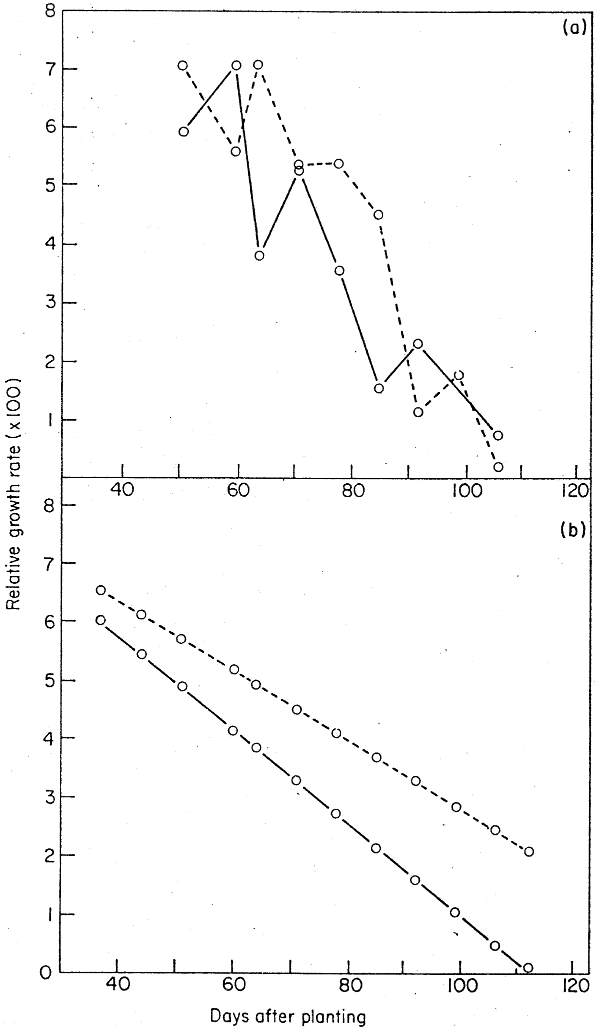

FIG. 6. Effect of two treatments on the change in RGR with time calculated by (a) Classical method, (b) Regression method (o—o covered, o---o uncovered).

be attributed to increase in self-shading. Maintenance of a better plant-water status also resulted in greater growth rates. Regression procedures permit better predictions of growth rates than do the classical methods when limited data points are available and the regression equation has been established.

Net Assimilation Rate is the net difference between the amounts of dry matter assimilated and respired. NAR, like RGR, has photosynthetic and respiratory components, and the relative importance of respiration increases with plant age. Figure 8 depicts the changes in NAR during the growing season. The effect of water stress on the soya bean plants in the covered plots is evident as NAR decreases with plant age. This agrees with the conclusions of Vaclavik (1967, 1969). The usefulness of the regression procedure is well illustrated in the case of the uncovered plots. NAR showed an increase 75 days after planting when the regression procedure was used, whereas it decreased with age in the case of the classical methods. The uncovered plots were irrigated 61 and 70 days after planting. The plants showed a dramatic recovery from stress as both leaf area and dry weight of the plants

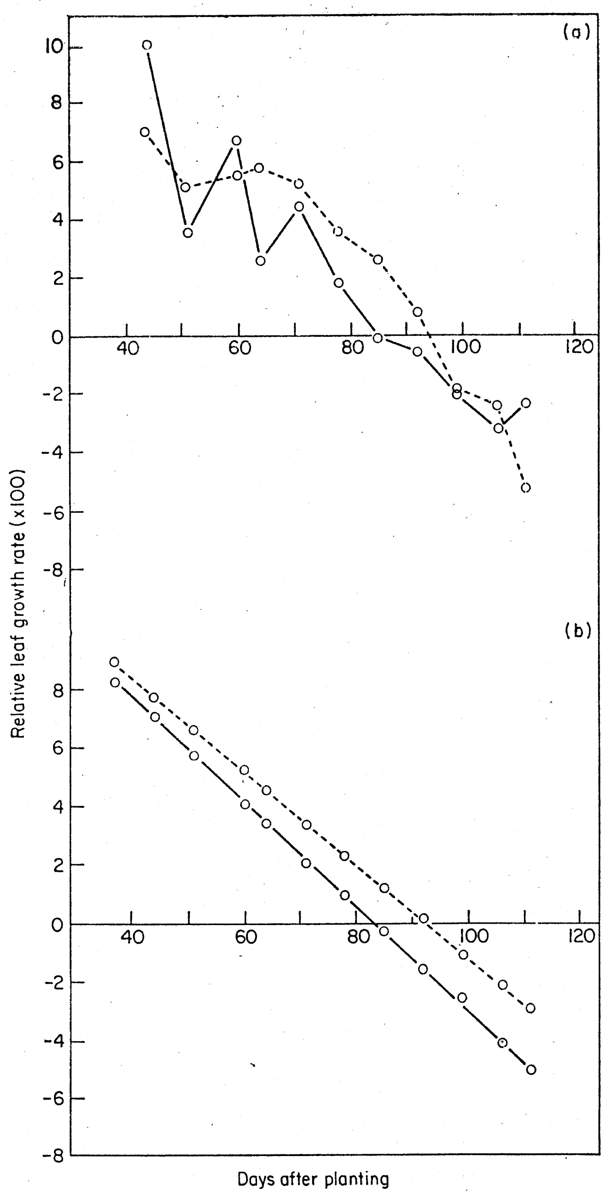

FIG. 7. Effect of two treatments on the change in RLGR with time calculated by (a) Classical method, (b) Regression method (o—o covered, o---o uncovered).

showed greater increases (Fig. 1). Leaf area started to decline 92 days after planting, whereas the dry weights increased until 100 days after planting and then levelled off. In as much as NAR is a net balance between respiration and photosynthesis, it is evident that the increase in NAR is not an artifact of the regression procedures, but is the result of accelerated production rate. Koller, Nyquist and Chorush (1970) interpreted the increase in NAR during the latter part of the growing season as a response of the photosynthetic apparatus to an increased demand for assimilates, which was due to the rapid growth of the seed.

The decrease in NAR values based on the classical method is misleading for two reasons.

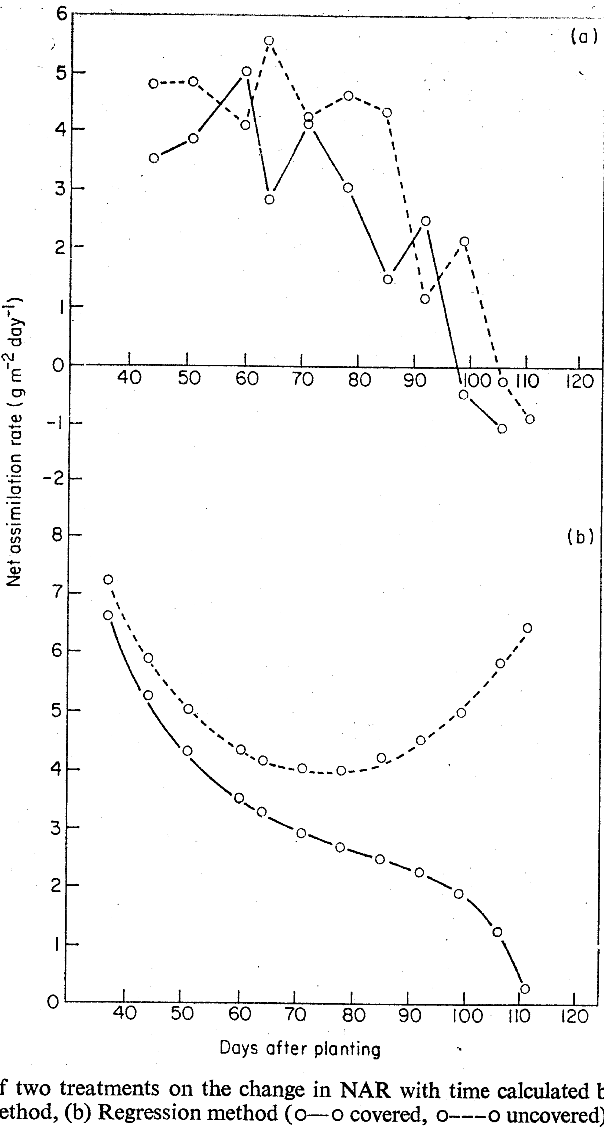

FIG. 8. Effect of two treatments on the change in NAR with time calculated by (a) Classical method, (b) Regression method (o—o covered, o---o uncovered).

The use of eqn (2) assumes linearity between leaf area and d. wt. Considerable departure from linearity was noticed in this investigation, and the actual relationship between A and W is not built into the equation as in the regression procedures.

Crop Growth Rate, as a function of time, is shown in Fig. 9. Uncovered plots maintained greater crop growth rates in both methods of growth analysis. Again, as in the case of NAR, the classical method showed a declining trend of CGC during the latter part of the growing season, as opposed to the increasing trend shown by the regression procedures. Per unit of ground area, uncovered plots maintained greater dry weights, whereas leaf area declined. As Milthorpe and Moorby (1969) and Koller *et al.* (1970) pointed out, photosynthetic activity is influenced by photosynthetic utilization. Eastin and Gritton (1969) also observed increasing CGR while leaf area was decreasing during the pod-filling stage of canning peas. Hence, regression procedures represent the actual situation, whereas the classical methods

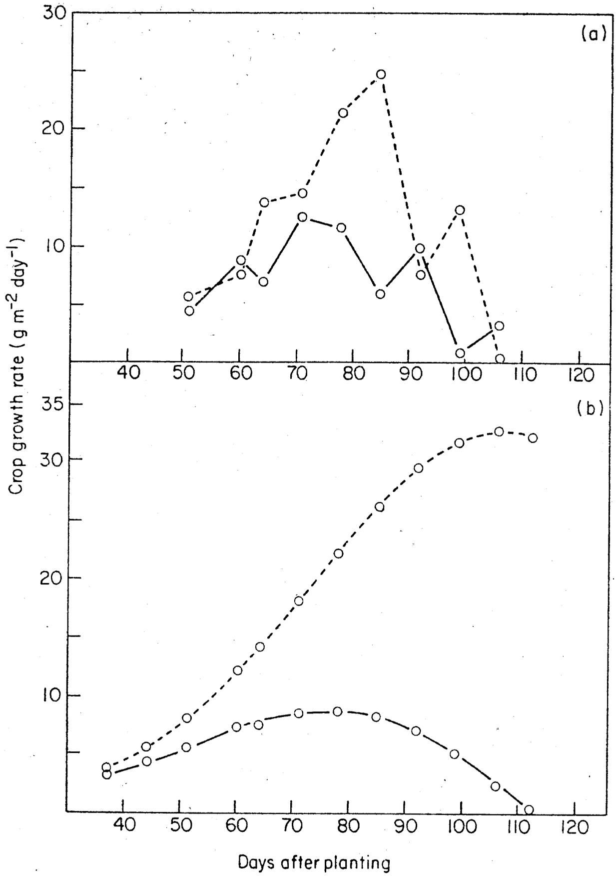

FIG. 9. Effect of two treatments on the change in CGR with time calculated by (a) Classical method, (b) Regression method (o—o covered, o---o uncovered).

used fail owing to the inappropriate use of the relationship between the leaf area and d. wt, and both can be biased by including leaves that are not assimilating.

Some statistical parameters that describe the effectiveness of the two methods of growth analysis are shown in Table 1. In all instances, use of regression procedures resulted in smaller variance, smaller standard error, and greater mean values as compared with the classical approach. Correlation coefficients between values calculated by using the two procedures reflect the underestimation of NAR and CGR by the classical methods. For RLGR, the calculatedv alues by the two methods are highly correlated. Hence, it is clear that, in the calculation of RGR, NAR and CGR, one has to pay attention to the exact relationship between leaf weight and leaf area and use the appropriate mathematical equation.

In the investigations reported here, the moisture stress pattern during 1976 showed a linear trend, as evidenced by the soil-water contents measured at weekly intervals. For this reason, multiple-regression techniques relating growth indices to weather parameters showed a better correlation between growth indices calculated according to regression procedures and soil-water contents. Low correlations were obtained between growth indices calculated according to classical methods and soil-water contents. This would not necessarily be the case, however, if the stress pattern under field conditions is more cyclic.

TABLE 1. *Statistical parameters comparing the two methods of growth analysis*

Growth Index	Method	Covered plots Variance	S.E.	Mean	r*	Uncovered plots Variance	S.E.	Mean	r*
NAR	classical	7·26	0·85	1·81	– 0·02	4·86	0·66	3·15	– 0·66
	regression	1·35	0·37	3·01		0·74	0·26	4·88	
RGR	classical	10·32	1·02	2·59	0·20	14·30	1·14	2·92	0·24
	regression	2·73	0·52	2·98		1·80	0·41	4·10	
RLGR	classical	17·64	1·27	1·80	0·93	16·39	1·22	2·24	0·95
	regression	16·24	1·22	0·87		12·83	1·08	2·30	
CGR	classical	26·03	1·61	5·87	0·77	89·75	2·86	9·01	0·06
	regression	4·32	0·66	6·56		21·26	1·24	14·16	

* Correlation between values calculated by the two methods.

Under such conditions, as Buttery (1969) pointed out, regression procedures would fail to show the short-term moisture-stress effects on growth indices.

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