PLANT FACTORS AFFECTING BETA
GAUGING OF LEAF-WATER STATUS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

By

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ACKNOWLEDGMENTS

The work reported in this thesis was carried out during a period of study leave granted by the Government of Ceylon. Financial support during the entire period of study, which was from 1959 to 1963, was provided by the Rockefeller Foundation, New York.

Profound thanks are also due Professors N. Holowaychuk and G. S. Taylor, and other members of the Staff of the Department of Agronomy of The Ohio State University; and to Dr. H. J. Mederski of the Ohio Agricultural Research and Development Center at Wooster, Ohio, under whose guidance and direction the work was done. Many others at Wooster, too numerous to mention individually, were exceedingly helpful in carrying out the work. Gratitude is expressed to all of those colleagues at Wooster.
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INTRODUCTION

Plants require a relatively high water potential and a high state of turgidity in the tissues for normal growth. Internal plant water balance is a function of several variables; namely, the moisture availability in the root zone, the evaporative demand of the foliar environment and any transpiration controlling factors that may exist within the plant itself. Although there is considerable published work on these three variables, the turgidity of internal water condition within the plant itself has not been extensively studied in relation to these variables. While the paramount importance of such studies is recognized, the practical difficulties in the measurement of the internal water status has mitigated against undertaking them. The development of methods for the measurement of the internal water status of plants would therefore greatly aid in our understanding of the moisture factor in plant growth. The present study is an attempt to further improve a method for continuously nondestructive monitoring of internal plant water balance.

LITERATURE REVIEW

Several methods of measuring and expressing the internal water condition of the plant exist and have been used by different workers. Lloyd (8) used water weight per unit area of leaf to express plant water
condition. Stocker (18) used the following leaf weight quantities to calculate the water status of the leaf:

\[
\text{Saturated fresh wt.} - \frac{\text{Field fresh wt.}}{\text{Saturated fresh wt.} - \text{Oven dry wt.}} \times 100
\]

Saturated fresh weight was taken as the weight of a leaf tissue after floating on a surface of pure water for a definite period of time and subsequent removal of surface water on the leaf. The terms field fresh weight and oven dry weight are self explanatory. The quantity thus calculated was termed the Saturation Deficit. Its value would be zero for a fully turgid tissue and numerically higher than zero for less turgid conditions.

Weatherly (22) adopted the quantity which he called Relative Turgidity (abbreviated as R.T.) defined as follows:

\[
\frac{\text{Field fresh wt.} - \text{Oven dry wt.}}{\text{Saturated fresh wt.} - \text{Oven dry wt.}} \times 100
\]

Relative turgidity takes a value of 100 for a fully turgid condition and is numerically lower for lesser states of turgidity. Besides introducing the relative turgidity concept, Weatherly also made extensive investigations on the experimental procedure for attaining the fully turgid condition. Temperature, light intensity and time period of flotation were among the factors that he investigated. These factors are of considerable importance, because respiration and photosynthesis also cause weight changes in a specimen of plant tissue. Barrs and Weatherly (2) have made further investigations of these factors.

Meyer and Wallace (12), and Meyer (11), used the concept of diffusion pressure deficit (D.P.D.) to express the internal water condition of plants. The expression of internal water condition as the diffusion
pressure deficit has one important advantage over other methods. The
energy status of soil moisture and atmospheric moisture can also be
stated in terms of the diffusion pressure deficit and moisture movement
in a soil-plant-atmosphere system may be readily determined. The main
factor which limits the extensive use of D.P.D. as an index of plant
water condition is the experimental difficulty of measuring it. The
principal method for measuring the D.P.D., involves the balancing of the
plant tissue D.P.D. against the D.P.D. of an aqueous solution of a
substance like sucrose or mannitol, using a series of solutions of
varying concentrations. The D.P.D. of the tissue is taken as being
equal to that of the solution in which the tissue neither gains nor loses
water. The loss or gain of water is detected by recording some
characteristic of the tissue or of the external solution, which undergoes
changes as the tissue gains or loses water. Among the characteristics
that have been used for this purpose are cell volume Ursprung and Blum
(20), tissue length Ursprung (19) and Lyon (1), tissue weight Meyer and
Wallace (12), and changes in concentration of the solution Ashby and
Wolfe (1), 1947. The D.P.D. of tissues might be estimated through
relative turgidity measurements if there exists a good relation between
these two quantities. Weatherly and Slatyer (23) have given some data
on the D.P.D./R.T. relationship for privet and tomato. They found that:
(a) the relationship was nonlinear, (b) the relationship was different
for the two plants, and (c) there was some variability even within each
species.

Other expressions which have been used for internal water condition
of plants are: (a) the water as a per cent of the dry matter, and (b)
the water as a per cent of the fresh weight. As general methods of expressing plant water condition, these are not satisfactory but in special cases they may be useful. Kramer (6) cites the expression of the water as a per cent of the dry weight in working with sugar cane in Hawaii. In this case Kramer mentions the fact that plant tissues of similar maturity were used. This is a necessary condition because changes in dry matter content which accompany maturation of tissue cause changes in the value of the percentage moisture content, even when no real change of moisture content has occurred. Diurnal changes of dry matter due to photosynthesis or respiration also cause changes in the moisture percentage. Sampling of tissue should, therefore, be made at fixed times of the day.

One of the difficulties of studying the internal water condition of plants, expressed in any of the above ways, is a suitable method of making frequent measurements without damaging the experimental plant. The problem is analogous to studying soil moisture without disturbing the experimental soil by frequent samplings. The existing methods of determining the internal water condition of plants is analogous to the methods of gravimetric determination of soil moisture. The advent of the continuous and nondestructive methods of determining soil moisture have made it possible to make great advances in all fields of experimental work in which soil moisture measurement is involved. It is conceivable that similar progress in investigation of plant water balance would follow if it were possible to make these measurements by continuous and nondestructive methods.
Box and Lemon (4) have reported on the use of an electrical resistance method for measuring plant moisture in cotton and sorghum. They reported some success with cotton, but little or no success with sorghum. They did not, however, investigate the relationship between electrical resistance and the R.T. of the plants, but only with the moisture level of the soil in which the plants were growing.

Namken and Lemon (13) have reported some further work using the electrical resistance method, using corn as the experimental plant. In this work some measurements of relative turgidity were made, but no definite relationship had been established between electrical resistance and relative turgidity. The authors mention that more detailed field and laboratory research is needed to determine whether the method is suitable for monitoring the internal water status of plants.

Mederski (10) has published the results of a continuous nondestructive method for monitoring RT and water content of soybean leaves by beta ray thickness gauging. In this method, he used the experimental leaf interposed between a Carbon 14 source of beta radiation and a Geiger tube detector. He found an almost linear relation between the R.T. of the leaf and the logarithm of the beta ray count. The correlation coefficient for the relationship varied from 0.93 to 0.99 for the sample of ten leaves that he used. Further, he found that the beta gauge provided a sensitive indication of the changing water status of an intact plant leaf as it responded to changes in soil moisture stress.

The principles of beta-gauging are well established and widely used in industry for thickness, density and concentration determinations (5). When matter of any kind is interposed between a beta radiation source and
detector the radiation passing through the matter and entering the
detector becomes increasingly attenuated as the mass per unit area
(mg/cm$^2$) increases. The combined effects of emission of beta particles
from a given nucleide, and the interaction of these particles with
matter produce an approximately exponential absorption relationship.
This relationship is expressed with sufficient accuracy for most
purposes by the familiar exponential absorption equation:

$$I = I_0 e^{-nx}$$

where:

- $I_0$ = the intensity of the incident unabsorbed beam.
- $I$ = the intensity of the emergent beam.
- $X$ = the thickness (mg/cm$^2$) of the absorber, and
- $n$ = the linear absorption coefficient of the
  absorber.

The application of the beta gauging technique for determination of
water content in plant leaves, depends on the fact that in any short
time interval a change in water content of the plant leaf may be repre­
sented by a change in the mass of water per unit area of the leaf
surface. This change in mass per unit area may be looked upon as a
change in thickness of water per unit area. When a leaf is interposed
between a source of beta radiation and a defector tube the intensity of
the radiation entering the detector tube will increase as the leaf water
content decreases. The leaf water content or some expression of the
condition of leaf water may be inferred from the observed count rate by
an appropriate calibration to be discussed later.
The present work was directed to obtaining information on the beta gauging technique of determining the internal water status of plants. Among the aspects on which further information was required before the method could be used effectively in pertinent research were the following:

(a) The influence if any of the intensity of beta source and of its position relative to the detector tube and the leaf specimen on the R.T. versus beta count relationship.

(b) The relationship reported by Mederski was found when fully turgid leaves were allowed to decrease in turgidity. One of the objectives of the research undertaken was to investigate possible hysteresis effects when leaves are subjected to water desorption and absorption cycles.

(c) Mederski found some degree of variation in the R.T. versus beta count relationship for leaves of varying age or degree of development. Studies were, therefore, made to determine the consistency of the relation of beta ray absorption by the leaves and the factors affecting it.

(d) The technique reported by Mederski employed detached leaves. Investigations were then undertaken to develop procedures whereby the leaves of growing plants could be monitored.
MATERIALS AND METHODS

The Beta Gauge

The essential components of the beta gauge are: (1) a beta ray source, (2) a Geiger tube, and (3) a scaler for detecting the Geiger tube pulses.

Amorphous, radioactive carbon-14 was used as the source of beta radiation. About 0.120 mc of carbon was used for preparing each source. This was deposited uniformly over a circle of filter paper by allowing it to settle out of a suspension of the carbon made up in water. Suction was applied to the filter paper to remove the water, as well as to deposit the carbon firmly onto the filter paper. When the filter paper with carbon was thoroughly dry, it was given a coat of lacquer adhesive to hold the carbon firmly in place. The paper was then fixed onto a plastic disc, with a diameter of about one inch and a thickness of about one-half inch.

The source and detector tube were fixed on a laboratory ring stand with the carbon source about half an inch from the mica window of the Geiger tube. In this position, a count rate of about 200,000 counts per minute was obtained, when there was no absorber between the source and the tube. The experimental leaf was supported between the source and the detector tube on a suitable movable platform mounted between the beta source and the detector window. The platform contained a hole larger than the diameter of the source and the tube window to allow unobstructed passage of radiation. It is important that the leaf or leaf sample be
considerably larger than the mica window of the Geiger tube to avoid radiation reaching the tube without passing through the leaf material.

In working with detached leaflets or cut leaf discs, for which the R.T. versus beta count relationship was desired, the Geiger tube was connected to a decimal scaler, and counts were taken over a suitable time interval, usually one minute or half a minute. In work with intact plants, where a fairly continuous record of counts was required to register turgor changes in leaves occurring over periods of a few to several hours, the Geiger tube was connected to a count rate meter, and the meter was connected to a millivolt strip chart recorder.

Measurement of R.T. and Beta Gauge Count

Relative turgidity was measured substantially by the method of Weatherly (22). Leaf discs or whole leaflets from soybean plants were used. The leaflets or discs were floated on distilled water held in covered petri dishes, for approximately 24 hours. At the end of this time, the leaflet or disc was taken out of the water and the excess water on the surface was quickly removed by blotting with absorbent paper. The leaflet or disc was then quickly weighed on a direct-reading balance to the nearest five-tenths of a milligram. This initial weight represents the fully turgid condition. This leaflet or disc was then immediately placed on the beta gauge platform and a count taken. Weighing the leaflet usually took five to ten seconds, and the count was usually taken over a half-minute or one-minute period. This gave the count associated with a practically fully turgid condition. By taking repeated weighings, and immediately counting, the counts associated with
decreasing levels of relative turgidity were determined. The linear regression lines of beta count versus R.T. were plotted on semi-log paper. Using half-minute counts and weighing and counting without pause, it was possible to obtain about five or six pairs of values between 100 and 90 percent relative turgidity, under the laboratory conditions in which the work was done. The temperature of the room in which the work was done was controlled and kept constant at 75° F., while the illumination was approximately 35-foot candles. Under these conditions, R.T. of the detached leaflets or discs reaches about 75 percent in about 20 to 30 minutes. After the first five or six counts, which were taken for half-minute intervals, succeeding counts were taken for one-minute intervals.

When replacing the leaflet or leaf disc on the beta gauge for counting, it is desirable to replace and orient it in the same position each time. This was done by indexing the margin of the tissue to coincide with the index margin on the leaf holder.

Influence of Beta-source Intensity on R.T. Versus Count Relationship

In the course of the work, it was necessary to dismantle the beta gauge component parts and reassemble them many times. For this reason the beta count with no absorber changed from one setting to the next. It was, therefore, necessary to know whether these changes in the arrangement of components of the beta gauge assembly brought about any changes in the relationship between relative turgidity and counts. For this purpose two sources of radiation (i.e., two different plastic plates with C 14 deposits) were arranged on the same laboratory ring
stand, so they could be brought over the detector tube alternately, each source to the same fixed position, respectively. The count with no absorber was different for the two sources.

A fully turgid leaf disc was weighed and counted under one of the sources and then weighed again and counted under the other source. The leaf disc was thus counted alternately under each of the sources, until enough measurements were obtained to plot a curve of R.T. versus count for each of the sources.

The above arrangement of the two C14 sources was tested also by determining the beta counts with varying thicknesses of filter paper placed between source and detector.

**Hysteresis Effects**

Experiments were carried out to determine the possible existence of hysteresis effects in the R.T. versus beta count relationship. The desorption relationship was first determined in the manner described earlier, by successive measurements when a leaf disc was allowed to decline to about 80 percent R.T. The same leaf disc was subsequently replaced on the surface of water in a petri dish for a few minutes, so it could absorb some water. It was then taken out, excess water removed, weighed and counted. It was then replaced in water for a somewhat longer period, taken out, excess water removed, weighed and counted. This process was repeated, each time keeping the leaf disc on water for a longer period than before, thus allowing the leaf disc to become increasingly more turgid. Finally, the leaf disc was kept in water overnight and the weight and count obtained finally being taken as the fully turgid condition with absorbent paper. This could produce some
deformation in the leaf tissues. There was no way of avoiding this, however, but great care was taken to handle the leaf disc as gently as possible consistent with adequate removal of moisture. Two attempts were made to overcome these difficulties; in one case the leaf disc was kept just above the water surface in a covered container, instead of on the surface. Under these conditions, however, water absorption was very slow and full turgor was not achieved even after 36 hours. The second approach to the problem was to increase the relative turgidity by passing leaf discs through a series of sucrose solutions of varying concentrations. This, likewise, was unsuccessful, because (a) the equilibration time was quite long, about three hours; and (b) in some cases the intercellular spaces of the leaf tissue appeared to fill up with sucrose solution and as a result there was considerable gain in weight, often exceeding the weight of the fully turgid leaf disc. The technique finally adopted was to first determine the desorption curve, and then successively float the tissue on water for short periods, weighing and beta gauging the leaf disc after each flotation.

Variation of the R.T. Versus Beta Count Relationship for Leaves of Varying Ages and Stage of Development

When using the beta gauge for determining the internal water status of a plant, it is essential to select a particular leaf for the measurement. Leaves of different maturities exist on a plant, such as soybean, and the object of this study was to find the nature of the relative turgidity versus beta gauge count relationship for leaves of different maturities of soybean plants. For this purpose, leaves of different maturities were obtained using the following procedures:
Procedure I. - Very young unexpanded leaves and all of the same stage of development as far as could be judged by eye, were selected from 36 plants growing in the field, one leaf from each plant. These leaves were marked for later identification by placing a very small plastic band around the petiole. Leaflets were sampled from the marked petioles at intervals of about five days, beginning with the stage when they were just large enough for beta gauging. Six leaflets were sampled at each stage. Leaves were ringed on June 28, the first leaflet sampling was taken on July 5, and the last on August 8. In one series of samplings, while leaves in the second series were marked on August 1. The latter sampling was initiated on August 7, and the last sample taken on August 23.

Procedure II. - In this method, leaflets for beta gauging were taken from leaves at different positions on a plant. Since the leaves of the soybean open in acropetal succession, leaves at different positions represent leaves of different maturities. Plants in the early pod stage of development were selected, and leaflets were taken beginning with the topmost leaf that had leaflets large enough for beta gauging. This topmost leaf was identified as position 1, and the next leaf below was position 2, and then position 3, and so on until position 7, which was usually the lowermost leaf that was in good condition. The leaf in position 8 was usually dried or otherwise damaged and was not suitable for beta gauging. Four different plants were sampled in this way and the R.T. versus beta gauge count relation was determined.
Beta Gauging of Field Plants

Two problems are encountered in beta gauging of field plants. One is the problem of calibrating a leaf of a field plant which has been used for beta gauging; i.e., establishing the R.T. versus beta gauge count relationship for that particular leaf. The second is that of variation, if any, of the relative turgidity among leaves on a plant. A few preliminary experiments were carried out on those two problems.

The work on the first problem was as follows: Two plants, one from each of two plots, were beta gauged for a period of four days continuously. The two plots were under different soil moisture regimes during that period. One plot was maintained near field capacity, by daily irrigation, while the other was not watered during the 4-day period, and the soil moisture level, therefore, decreased from field capacity initially to near wilting percentage at the end of the 4-day period. One of the leaves, located at midposition on the stem from each of two experimental plants, one from each plot, was firmly held in a fixed position between the beta-ray source and the Geiger tube on a suitable platform. Tabs of masking tape were applied at several points along the leaf edge to hold the leaf in place. The Geiger tubes for each of the two experimental leaves were connected to a rate meter through a switching mechanism which connected alternately the Geiger tubes to the rate meter. The switching device was so adjusted that each of the tubes was connected to the rate meter for a period of about eight minutes during every 30-minute interval. In order to reduce the fluctuations in count to a minimum, the time interval constant of the rate meter was set to its

1Nuclear of Chicago. Model 1620B.
maximum, which was 40 seconds. The instrument took about five such time intervals, or about 200 seconds to reach equilibrium. Because of this, only the last four minutes of each 8-minute record was used to ascertain the equilibrium count rate. The counting range of the rate meter was judiciously chosen, for maximum sensitivity, compatible with a range that was adequate to include the highest count rates likely to be encountered in either of the experimental plants. The rate meter was connected to a millivolt strip chart recorder. Suitable housing was provided for the rate meter and recorder to protect them from the weather.

During the period of beta gauging, relative turgidity determinations were made using leaf samples from some of the other plants in the two plots. This was done on 14 occasions to verify the beta gauge data. At each time two samplings were taken from each plot, each sampling consisting of six leaf discs taken at random from the plants in the plot. The six leaf discs were placed in a stoppered weighing bottle for subsequent weighing in the laboratory. The mean R.T. of each of the 14 samplings taken from each plot was compared with the R.T. values indicated by the beta gauge data for corresponding time periods.

At the end of the period of beta gauging the leaves gauged were calibrated in the laboratory by the following procedure: Additional strips of masking tape were fixed to the leaf so as to leave a roughly square area of leaf exposed. This exposed square area of leaf included the portion which had been directly under the carbon source, and also extended about a tenth of an inch beyond. This square segment of the

\[\text{Sargent, Model MR.}\]
leaf was carefully cut out along the inner edge of the strips of masking tape with a sharp blade for subsequent operations. The square piece of leaf was made fully turgid by floating on water, and its R.T. versus beta gauge count relationship was obtained as described previously. During calibration the geometry of the beta gauge and the position of the leaf sample on the gauge were identical to the geometry of the leaf position while gauging was done in the field.

The second problem of possible variation in relative turgidity among leaves on a plant required investigation, because in beta gauging it is usually not convenient to gauge more than a single leaf on a plant at a time. It is necessary, therefore, to know how representative the leaf is of all the other leaves on the same plant. With this in view, a simple study was made of the relative turgidities of the leaves of the upper third, middle third, and lower third of plants growing in the field. This was done at three different times during the day; early morning, forenoon and afternoon. Those times were chosen so as to secure comparisons among different leaves on the plant as they pass through diurnal variations in turgor. At each time, six plants were selected and from each plant nine leaf discs, three representing the upper third of the plant; three, the middle third and three, the lower third were taken for R.T. determinations.
RESULTS AND DISCUSSION

Influence of Beta Source Intensity on R.T. Versus Beta Count Relationship

For a given geometry the beta source intensity (Io) is indicated by the count rate when no absorber is present between the source and the detector tube. Expressed in cpm (counts per minute), this intensity Io for each of the two sources used, A and B, was as shown below:

Source A - 80,000 cpm
Source B - 200,000 cpm

The influence of source intensity on the quantity of beta radiation entering the detector tube after passing through an absorbing medium was evaluated by interposing varying thicknesses of absorber in the pathway. The results, shown as plots in Figure 1, indicate that while the quantities of radiation entering the detector differ according to source; there is a similar pattern of change for each source with varying thicknesses of the absorber. This change in radiation, received by the detector from each source, shows a linear relationship:

\[ y = a + bx, \]

where \( y \) = relative thickness of absorber (mass/unit area), and \( x \) = detector beta count in \( \log_{10} \) cpm.

The terms "a" and "b" are constants. There is a shift in the intercept "a" on the x axis, but the count rates of change "b" indicated by the plots for each source are alike. A similar evaluation of the influence of beta source intensity was made using a leaf disc in which
the R.T. was allowed to vary as the interposed absorbing medium. The plot of observations shown in Figure 2 revealed a relationship similar to that where filter paper was the absorber. It is evident that while the intensity of the beta source affects the magnitude of transmitted radiation received by the detector, the rate of change in transmitted radiation due to interposed absorption is similar. These are useful results because a certain amount of flexibility is available in setting up the beta gauge.

**Hysteresis Effects**

Fifteen leaf discs were investigated. In all cases a leaf disc was taken from a plant and allowed to lose water by evaporation, while the corresponding data for desorption beta count were obtained until the R.T. decreased to 80 percent. Subsequently, the leaf disc was allowed to resorb water by successive stages to full turgidity, with concurrent beta gauging at each stage. In all cases the desorption and absorption curves were not essentially different. Plotted data for three of the leaf discs, shown in Figures 3 (A and B) and Figure 4, are presented as examples. There was no evidence of hysteresis. These data indicate that the R.T., inferred from beta count rate, is not affected by whether a given R.T. is brought about by either a gain or a loss of water.
Fig. 1. Influence of beta ray intensity on the relation between thicknesses of absorber and beta count rate.
Fig. 2. Influence of beta source intensity on the relation between relative turgidity of a leaf disc and beta count rate.

Beta counts (in thousands) per minute. Log10 Scale.

Beta Source A

Beta Source B
Fig. 3. Relation of beta count rate to relative turgor of leaflets A and B with desorption and resorption of water.

- x - Resorption cycle.
- o - Desorption cycle.
Fig. 4. Relation between relative turgidity of leaflet and beta count rate with resorption and desorption of water.

- x - Resorption cycle.
- o - Desorption cycle.
Variation of R.T. Versus Beta Count Relationship
For Leaves of Varying Age or State of Development

The data, obtained on the first series of samplings using Procedure I, are shown as plots in Figures 6 through 10. In Figure 5 the individual data values are shown as plotted points to indicate the degree of scattering of points on the R.T.-beta count curve. Since the degree of scatter was similar in all cases, the plotting of individual values observed was omitted in Figures 6 through 10 for clarity, and only the resultant curves are shown. As evident in Figure 5, the curve for the very young leaves, i.e., those leaves that were sampled on July 5, had a much steeper slope than that of the curves obtained for leaves that were taken later. This indicates that in the case of the very young leaves there is a relatively small change in log of beta count per unit change in R.T. The beta gauging method is, therefore, not very precise for detecting variation in R.T. in very young leaves. More precision was obtained for the leaves taken later in this series.

The observations made on the leaves taken on July 5, July 15, July 30, August 3 and August 8 are represented by Figures 6 through 10 respectively. All of the resultant plots, shown in the latter figures, exhibit an appreciable increase in the slope of the lines relating R.T. to log of beta count. The variation in the slope of the lines amongst samples taken within a date is as large as the apparent variation in slope for samples taken at different dates. The large variance observed within samples makes difficult the determination of change in slope with leaf age.
Fig. 5. Relative turgidity versus beta count relation of six leaves sampled July 5.
Fig. 6. Relative turgidity versus beta count relation of seven leaflets sampled July 10.
Fig. 7. Relative turgidity versus beta count relation of seven leaflets sampled July 15.
Fig. 8. Relative turgidity versus beta count relation of six leaflets sampled July 30.
Fig. 9. Relative turgidity versus beta count relation of six leaflets sampled August 3.
Relative turgidity versus beta count relation of five leaflets sampled August 8.
The results obtained by Procedure I for the second series of leaf samplings are shown in Figures 11 through 16, and are summarized in Figure 17. In these figures the abscissa represent the logarithm of the difference in beta counts, obtained at a given R.T. and that at 100 percent R.T. This has been done by plotting the combined data for all leaves of each date as a single curve and thus permit a statistical comparison between leaflets of increasing age. In each of the figures 11 through 16 the calculated regression line was drawn incorporating the data from six leaf discs. To facilitate easier visual comparison, all of the data plotted as regression lines in Figures 11 through 16 were combined and shown by a single curve in Figure 17. If the equation for each line is written as \( y = bo - b_1X \), where \( y = \log_{10} \) of the difference in counts per minute, and \( x = R.T. \) the \( b_1 \) values of the six regression lines in Figures 11 through 15 are as follows:

<table>
<thead>
<tr>
<th>Line</th>
<th>Leaflet age (in days)</th>
<th>( b_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>98.6</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>67.8</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>57.5</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>52.1</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>53.0</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>63.4</td>
</tr>
</tbody>
</table>

The slope of Line 1 is significantly different from those of the remaining five, while the slopes of Lines 2 through 6 are not significantly different. These data indicate that in young developing soybean leaflets less than eight days of age, the relation of beta ray attenuation to R.T. is not constant but transitory. Because of the
Fig. 11. Relative turgidity versus change in beta count rate relation of leaflets sampled August 7, Series 2. Beta count change = $\log_{10}$ of difference (cpm at prevailing R.T.-cpm at 100% R.H.)
Relative Turgidity

Fig. 12. Relative turgidity versus change in beta count relation of leaflets sampled August 9, Series 2. Beta count change = $\log_{10}$ of difference (cpm at prevailing R.T. - cpm at 100% R.T.)

$\log_{10}$ of change in beta count rate in cpm.
Fig. 13. Relative turgidity versus change in beta count rate relation of leaflets sampled August 13, Series 2. Beta count change = $\log_{10}$ of difference (cpm at prevailing R.T. - cpm at 100% R.T.)
Fig. 14. Relative turgidity versus change in beta count rate relation of leaflets sampled August 16, Series 2. Beta count change = $\log_{10}$ difference (cpm at prevailing R.T. - cpm at 100% R.T.).
Log₁₀ of change in beta count rate in cpm

Fig. 15. Relative turgidity versus change in beta count rate relation for leaflets sampled August 20. Beta count change = Log₁₀ of difference (cpm at prevailing R.T. - cpm at 100% R.T.)
Relative turgidity versus change of beta count rate relation for leaflets sampled August 23. Beta count change = $\log_{10}$ of difference (cpm of prevailing R.T. - cpm at 100% R.T.).
Fig. 17. Composite of Figures 11 through 16. Showing relative turgidity versus change on beta count rate relation for leaflets sampled at six stages of maturity as shown by sampling dates in Series 2. Beta count change = $\log_{10}$ difference (cpm at prevailing R.T. - cpm at 100% R.T.)
lack of constancy young leaflets are not suitable sites for extended periods of gauging. On the other hand, leaflets 12 days old or older exhibit similar slopes when observations are plotted and hence should be acceptable for extended gauging.

The data for the length and breadth of leaflets of the leaf samples gauged in Procedure 2 are shown as plots in Figures 18 and 19. These data show that the constancy of slopes of plotted lines when leaflets are 12 days old or older is associated with a cessation of increase of leaf size. It is concluded, therefore, that fully expanded leaflets appear to be satisfactory for gauging to determine the R.T. - beta count relationship.

In Procedure II used in investigating the variation in the R.T. - beta count relationship for different leaves the leaflets were taken from leaves at different positions on a plant. These different positions represent differences in age and presumably, but not necessarily, differences in stage of maturity. These leaflets may vary in age but some of different ages may be equally mature. As indicated in the outline of this procedure the leaves were taken from the upper, the middle and the lower portions of a plant. The leaf positions were identified by numbers with 1 referring to uppermost leaves that were large enough for beta gauging and number 7 the lowermost. Figure 20 shows a plot of the data for leaves from position 1, and Figures 21 and 22 represent the data for positions 3 and 5, respectively. The data for leaf positions 2, 4, 5 and 6 are not shown since they are similar to those for leaves from positions 1, 3 and 5. As the plotted data in Figures 20, 21 and 22 indicate even the youngest leaves (i.e., leaves
Fig. 18. Change in length and width of developing soybean leaflets with time (Series 1).
Fig. 19. Change in length and width of soybean leaflets with time, Series 2.
Fig. 20. Relative turgidity versus beta count relation for four soybean leaflets taken in position 1 on a plant.
Fig. 21. Relative turgidity versus beta count relation for four soybean leaflets taken in position 3 on a plant.
Fig. 22. Relative turgidity versus beta count relation for four soybean leaflets in position 5 on a plant.
from position 1 do not show any consistent difference or trend in slope from leaves taken at the other positions. The curves obtained from the four leaflets taken at each position show as much within sample variation as there is between samples. The variance in beta count rate at 100 percent R.T. among leaflets is an indication of differences in total mass per unit area.

These results indicate that if one used fully expanded leaflets for measuring R.T. by beta gauging, it is possible to gauge a leaflet for several days and then at the end of this period the experimental leaflet can be detached from the plant and calibrated to obtain the R.T.-beta count relation. This can be assumed to be valid for the week of the experimentation since the change in age of the leaflet during that week apparently has no effect on the relationship.

Figures 20 through 22 show the curves obtained for leaves removed from different heights of insertion. The variance in count rate at 100 percent R.T. among leaflets is an indication of differences in total mass per unit area.

Leaf Disc Area Shrinkage and Preferential Desorption of Water at Periphery

The studies on the variation of R.T. versus beta gauge count relationship indicated no detectable variation between fully expanded leaflets. Nevertheless, as a corollary to that work, some experiments were carried out to investigate whether lateral shrinkage of leaf discs occurred and whether there was preferential desorption of water at the periphery of leaf discs when a fully turgid leaf disc loses water by evaporation. The occurrence of either of these phenomena could lead
to a relatively small change in count rate when a leaf disc that is losing water by evaporation is gauged. This method of monitoring R.T. changes would have maximum efficiency in cases where loss of water from a leaf disc is accompanied by a uniform decrease in the thickness of the disc without any surface area shrinkage and without any preferential decrease in thickness at the periphery of the disc.

Lateral shrinkage was investigated by making use of the fact that if leaf shrinkage takes place when a leaf loses water by evaporation, then a leaf disc of the same diameter cut from a fully turgid leaf should have less dry weight than would a disc of the same size cut from a less turgid leaf.

In one experiment, the leaf discs which had been floated on water for 24 hours were taken out individually, excess water removed and allowed to dry for specified time intervals. Then an inner disc was punched out of each. This smaller disc was oven dried and weighed. Seven time intervals were used and a sample of six discs were used each time. The data from these experiments are given in Table 1, and the relation of the mean dry weights to time is shown in Figure 23.
Fig. 23. Weight of leaf discs as a function of drying time.
TABLE 1

Dry weights of leaf discs cut from leaf tissue after loss of water from a fully turgid condition

<table>
<thead>
<tr>
<th>Time in Minutes</th>
<th>Dry weight in grams of each of six discs</th>
<th>Mean dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>.0161 .0162 .0128</td>
<td>.0135</td>
</tr>
<tr>
<td></td>
<td>.0130 .0120 .0106</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>.0131 .0130 .0141</td>
<td>.0145</td>
</tr>
<tr>
<td></td>
<td>.0138 .0165 .0165</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>.0163 .0154 .0156</td>
<td>.0146</td>
</tr>
<tr>
<td></td>
<td>.0152 .0125 .0129</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>.0134 .0133 .0142</td>
<td>.0141</td>
</tr>
<tr>
<td></td>
<td>.0139 .0156 .0141</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>.0168 .0172 .0137</td>
<td>.0157</td>
</tr>
<tr>
<td></td>
<td>.0137 .0162 .0164</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>.0168 .0178 .0161</td>
<td>.0168</td>
</tr>
<tr>
<td></td>
<td>.0156 .0173 .0172</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>.0176 .0158 .0164</td>
<td>.0164</td>
</tr>
<tr>
<td></td>
<td>.0148 .0171 .0167</td>
<td></td>
</tr>
</tbody>
</table>

In an analysis of variance of the data, the calculated "F" value exceeded the tabulated value at one percent probability level, indicating a significant increase in dry weight per unit area with a decrease in leaf turgor. This increase in weight per unit area or turgor decrease is evidence that leaf area decreases as turgor decreases.

 Preferential desorption of water at the periphery of leaf discs was investigated by making use of the fact that if fully turgid leaf discs are allowed to lose water for a few minutes, and an inner disc is cut out
centrally, then the moisture percentage of the inner disc would be higher than that of the outer ring, if there is preferential desorption of water. In the experiment, 22 leaf discs were floated on water for 24 hours. At the end of this time, the discs were taken out singly, excess water removed from the surface and allowed to dry for a fixed time interval of five minutes. An inner disc was then cut out from each, leaving an outer ring of tissue. For eleven of the discs, the inner disc was weighed and the green weight of the ring obtained by difference. For the remaining eleven, the ring was weighed and the inner disc weight obtained by difference. This was done because there was a slight time lag between weighing the initial large disc and weighing the inner disc or the outer ring. If the inner disc is weighed, then this overestimates the green weight of the ring, and vice versa.

The mean moisture percentages, on a dry weight basis for each of the two sets of eleven disc samples, are given below:

<table>
<thead>
<tr>
<th></th>
<th>Inner disc</th>
<th>Outer ring</th>
<th>Average for 22 discs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner disc</td>
<td>416.5</td>
<td>440.1</td>
<td>428.3</td>
</tr>
<tr>
<td>Outer ring</td>
<td>423.5</td>
<td>415.0</td>
<td>419.3</td>
</tr>
<tr>
<td>Whole disc</td>
<td>418.8</td>
<td>432.0</td>
<td>425.4</td>
</tr>
</tbody>
</table>

It is clear from the mean values given above, that there is no preferential desorption of water at the periphery of leaf discs. The mean moisture percentage is slightly greater where the inner disc was weighed, but in this case the weighing sequence tended to bias the results in favor of a greater moisture percentage for the outer ring.
Where the outer ring was weighed, the results were biased in favor of a greater percentage for the inner disc; but in this case, the difference in moisture percentages was much greater than when the inner disc was weighed. Thus, there is no evidence to indicate the possibility of preferential desorption of water by leaf discs.

**Beta Gauging of Field Plants**

A technique which would permit monitoring of R.T. of growing plants without requiring detachment or destruction of parts of a plant would have considerable usefulness. It would be especially useful if this monitoring can be performed in the field on plants in situ. The discussion following reviews the results obtained by beta gauging of field plants.

The technique used involved two procedures which have been discussed earlier under Methods and Materials. Briefly, these procedures consisted of:

1. Determination of the R.T.-beta count relationship by monitoring leaves in situ in the field and, after detachment, by beta gauging in the laboratory.

2. Determination of R. T. by beta gauging of leaves in situ in the field with concurrent sampling of leaves of contiguous plants for beta gauging in the laboratory.

The data obtained by the first procedure are shown in Figure 24. While there is somewhat greater scatter in values obtained by gauging in the field at the time of sampling, in comparison to those observed in the laboratory, there is good agreement between these two measurements.
Fig. 24. Relation between relative turgidity as measured by direct sampling of leaves and by beta gauge technique.

- x - Leaf turgor inferred from beta gauging in laboratory.
- o - Leaf turgor inferred from beta gauging in field at time of sampling.
Figure 25 represents a graphical comparison of the R.T. values obtained by the two procedures. It is evident that the degree of correlation is reasonably good. Lack of better agreement may be attributed to the fact that the R.T. of the gauged plants was actually different from the mean R.T. of the plants from which the samples were taken.

Beta gauging of plants in the field over extended periods of time has made it possible to evaluate diurnal variations in R.T. The data plotted in Figure 26 show the diurnal changes in R.T. of leaves of plants of two experimental field plots, one which was irrigated. In this graph the R.T. values plotted on the ordinate have been calculated from the calibration curve of Figure 24. The progressive decrease of turgidity as the soil in the unirrigated plot dried is shown very well. The measured soil moisture levels of the two plots are given in Table 2.

Table 2
Soil moisture content of irrigated and nonirrigated plots expressed as percent by weight - dry weight basis

<table>
<thead>
<tr>
<th>Soil Depth Date</th>
<th>Time (p.m.)</th>
<th>Irrigated plot Depth 0-6&quot;</th>
<th>6-12&quot;</th>
<th>Unirrigated plot Depth 0-6&quot;</th>
<th>6-12&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 31</td>
<td>4:00</td>
<td>22.4</td>
<td>21.1</td>
<td>15.0</td>
<td>12.0</td>
</tr>
<tr>
<td>August 1</td>
<td>4:00</td>
<td>28.1</td>
<td>25.1</td>
<td>12.0</td>
<td>11.4</td>
</tr>
<tr>
<td>August 3</td>
<td>4:00</td>
<td>20.8</td>
<td>20.2</td>
<td>10.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

With regard to the problem of possible variation in R.T. between leaves on the same plant, the results of the investigation mentioned in the previous section, are given in Table 3. The results show that
Fig. 25. Relation between relative turgidity (R.T.) as measured by direct sampling of leaves and by beta gauge technique.
Fig. 26. Diurnal changes of relative turgidity in field plants under irrigated and unirrigated conditions, as determined by the beta gauge technique.
the upper, and consequently the younger leaves of a plant have a slightly lower R.T. than found in the middle and the lower leaves. The difference is statistically significant at mid-day when the general level of turgidity of the whole plant is itself low. The difference is statistically significant in the forenoon period and in the morning, when turgidity is relatively higher. Therefore, presumably when the R.T. of the whole plant is near 100 percent, it may be supposed that all parts of a plant would be more or less at the same relative turgidity.

Table 3
Relative turgidity variation between the leaves of the upper middle and lower parts of the plant shoot

<table>
<thead>
<tr>
<th>Sampling Time</th>
<th>Relative turgidity of the leaves of the named part of the plant. Mean values of a sample of six plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
</tr>
<tr>
<td>August 29 10:30 a.m.</td>
<td>86.3</td>
</tr>
<tr>
<td>August 29 2:00 p.m.</td>
<td>83.6</td>
</tr>
<tr>
<td>August 30 8:00 a.m.</td>
<td>91.0</td>
</tr>
</tbody>
</table>
SUMMARY AND CONCLUSIONS

The work reported here consists of studies of a method of continuously monitoring the internal water status of plants, introduced and reported earlier by Mederski (10). The method consists of interposing a leaf between a source of beta radiation and a Geiger tube detector, and measuring the beta radiation traversing through the leaf at different states of turgidity of the leaf. A linear relationship exists between the R.T. of the leaf and the logarithm of the count rate measured by the Geiger tube. In the present work, the following further information was obtained:

(1) In a linear relation of the form $Y = a + bx$, where

$Y$ is relative turgidity

$X$ is the $\log_{10}$ of the count rate

$a$ is the intercept

$b$ is the slope of the curve.

The slope of the curve remained unchanged when beta sources of different intensity were used; intensity in this context denoting the count rate obtained when there is no absorber interposed between the beta source and the Geiger tube.

(2) The slope of the curve relating R.T. remained unchanged after the leaflets were more than 8 days of age. The slope constancy is necessary condition for using the beta gauge technique to measure R.T. in plants. If the beta gauge count rate has been measured for a leaf attached to a
plant, through a period of several days, and then it is detached and a relative turgidity versus count rate calibration obtained; this calibration can be assumed to have prevailed for the period of the measurements.

(3) Very young leaves, however, had an R.T. versus count rate relationship in which the curve was steeper than for mature leaves; i.e., the count rate increased relatively less as R.T. decreased from a fully turgid condition to less turgid levels. Some experimental work was done on this problem and it indicated that this may be due to the occurrence of lateral shrinkage of the leaf tissues as a disc of leaf tissue changes from a fully turgid condition to less turgid levels.

(4) The R.T. versus beta gauge count rate relationship obtained by progressive increase in turgidity of a disc of leaf tissue from less turgid conditions to fully turgid conditions, was more or less coincident with the relationship obtained by progressive decrease in turgidity from a fully turgid condition to less turgid conditions. There was no evidence of hysteresis.

(5) In beta gauging of field plants it was found that the beta gauge technique was satisfactory for registering changes in relative turgidity.
BIBLIOGRAPHY


