FIELD USE OF RECORDING VISCOUS FLOW POROMETERS

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I. INTRODUCTION

The fundamental idea behind mass flow (or viscous flow) porometry is quite simple. We reason that if we try to force a given quantity of air, or any other gas, through a leaf, entering through one surface and exiting through the other, it will be easier to accomplish this if the stomata are open than if they are closed. There are several basic ways in which this concept may be used to estimate the degree of stomatal opening. To obtain measurements in real resistance (or conductance) terms it is necessary to have some measure of the flux of gas through the leaf as well as the pressure head driving that flux.

Over the years a wide array of ingenious devices has been developed for that purpose and correlations demonstrated between mass flow resistance, diffusive resistance, and direct measurements of stomatal aperture. Most of these devices are adequately reviewed by Hsiao and Fischer (1975), Meidner (1981) and Slavik (1974) and we will say no more about them except that generally they are not well suited for both continuous monitoring of stomatal aperture and use in field situations.

If it is desired to obtain only relative measurements of stomatal opening then it is necessary to measure only the flux or the pressure head or the rate of change of either. The requirements for a device to make such measurements are

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much less stringent. Therefore such devices are more adaptable to continuous monitoring in a field situation. This is in fact the approach we have used for monitoring stomatal opening as a means of detecting plant water stress.

In this paper we will describe equipment and the data analysis used to relate the porometer measurements to stress levels, and compare this technique with more conventional measures of water stress.

II. EXPERIMENTAL PROCEDURES

The porometer cup (Figure 1) was a permanently attached type which consisted of a soft rubber cushion appressed to the abaxial surface of the leaf. The cushion was connected to an air supply through a very fine metering valve and the gas pressure at the lower surface of the leaf was sensed with an electronic pressure transducer. The cup was held in place by a Plexiglas plate placed on the upper surface of the leaf with an aperture cut in it to allow the free passage of gas. A CO_2-free mixture of nitrogen and oxygen was used as the gas supply to the porometers. The pressure was regulated to a nominal head of 0.7 kPa and the supply was fed simultaneously to an array of porometers (Figure 2). Pressures measured at the lower surface of the leaf ranged from nearly zero, when the stomata were fully open, to the line pressure when they were closed to the maximum extent possible. The pressure readings from each leaf were normalized to the maximum pressure read during a 24-hr period to allow for variability in installation and individual leaf differences, although these differences tended to be small. The photosynthetic photon flux density (PPFD) was the only other environmental parameter necessary for using the porometers as stress detectors since we were primarily interested in the stomatal response to light. A more detailed description of the system is given by Fiscus et al., (1984a).

Typical data are shown in Figures 3 and 4 where the normalized pressure readings are expressed as relative stomatal closure, the higher pressures indicating tighter closure. Figure 3 includes data taken on the same day from plants under 3 different irrigation treatments. Figure 3A shows the response from a well watered plant, 3B from a plant in the intermediate stages of water stress, and 3C from a plant that is stressed to the point of complete stomatal closure. The plant represented in Figure 3C still is capable

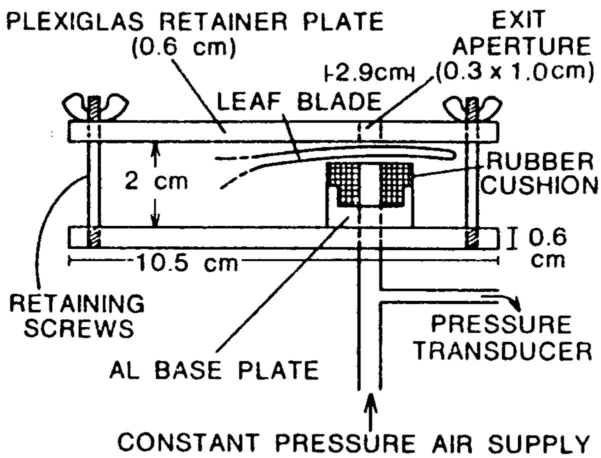

Figure 1. Detail of the mass flow porometer.

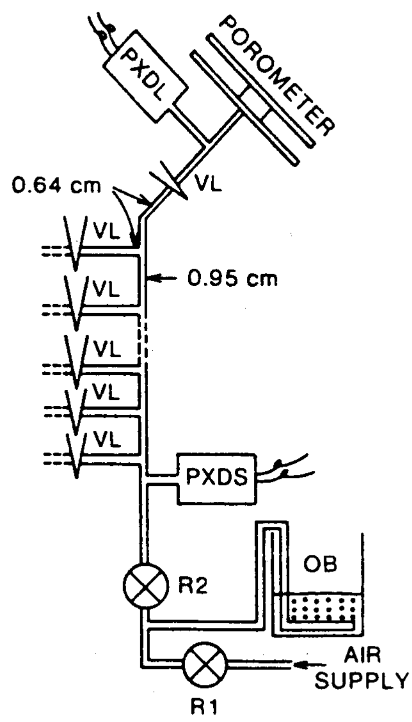

Figure 2. Manifold system for driving several porometers from the same air supply. The needle valves are set so that this resistance to flow is much larger than the leaf resistance over most of the range of opening.

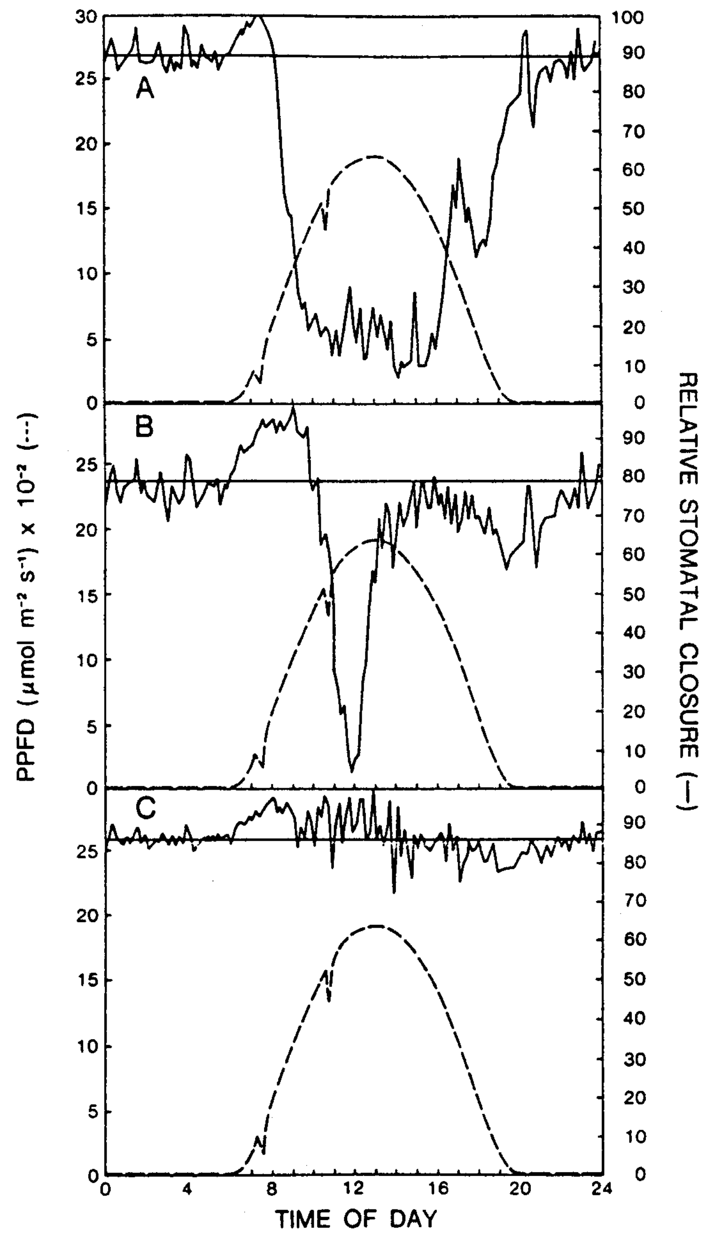

Figure 3. Typical data from three irrigation treatments on the same sunny day. Solid curves are the normalized porometer pressure readings and the dashed curve is the light intensity. Solid straight lines are the baselines.

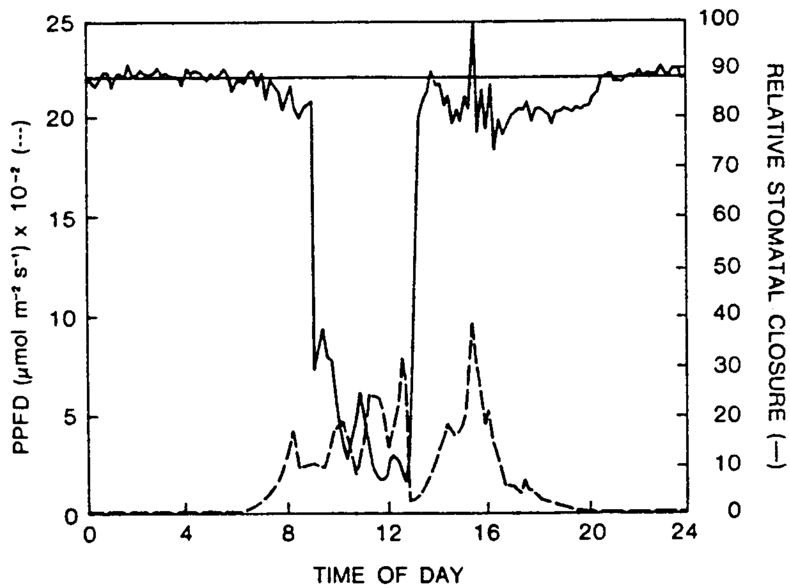

Figure 4. Typical data from a well-watered plant on an overcast day.

of recovery but obviously it is unable to fix much CO_2 under these conditions and will be damaged irreversibly if the stress is allowed to persist.

Figure 4 shows the response from a well watered plant on an overcast day and illustrates the reason why we have to deal with the stomatal response to light as the stress indicator and not just the degree of stomatal opening on any particular day. In this instance the stomata do not stay open during the day, not because the plant is stressed but because there is not enough light to keep them open. Simply monitoring relative stomatal opening therefore is not enough since data from cloudy days might be interpreted as advanced levels of stress. Also, as can be seen in Figures 3A and 3B, single measurements of the more conventional water status indicators, as well as mass flow porometry, taken at the wrong time of day (noon for example) might lead one to believe that both of those plants (Figures 3A and 3B) were equally well off. It is for these reasons that we go to the trouble of integrating the light and the relative stomatal opening each day. The integration of light is performed very simply by summing the intervals under the PPFD curve for each day. This sum is called the integrated light intensity (ILI).

The relative stomatal opening is integrated by taking the average of the readings between midnight and dawn and using that value as a baseline. The area below the baseline (Figures 3 and 4) is then summed over the 24 hr period and expressed as the integrated stomatal opening (ISO) for that day. Values of ISO are plotted against the corresponding values for ILI (Figure 5) and a boundary line drawn to represent the maximum possible values for ISO at any measured level of ILI.

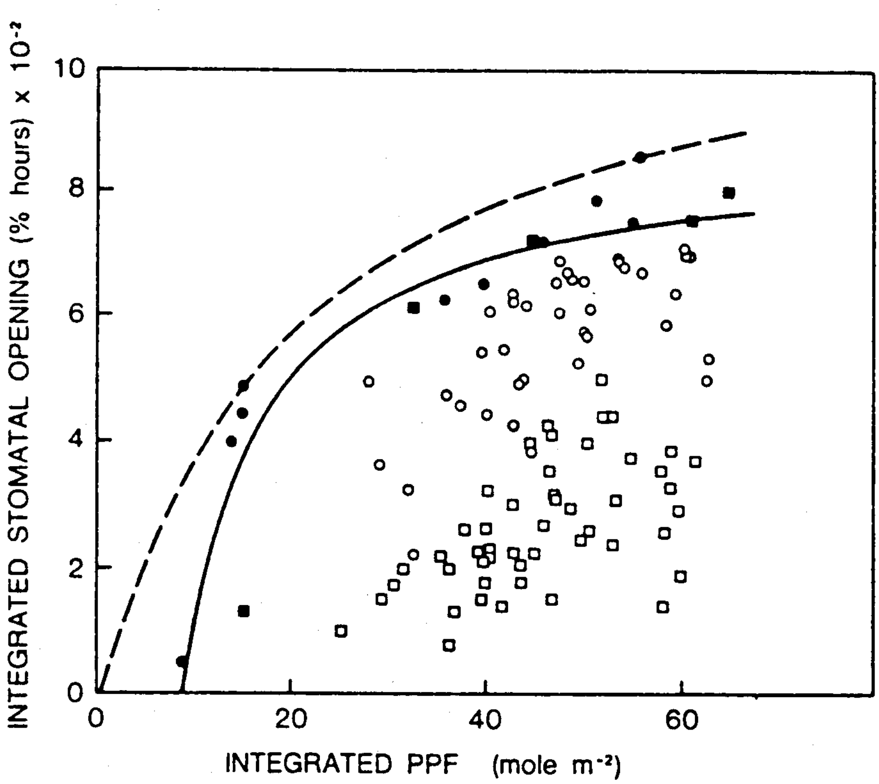

Figure 5. Plot of ISO vs. ILI. Solid line is the boundary line calculated from a hyperbolic regression of points near the maximum. Dashed line is the boundary ,line calculated from the double reciprocal plot (Figure 6).

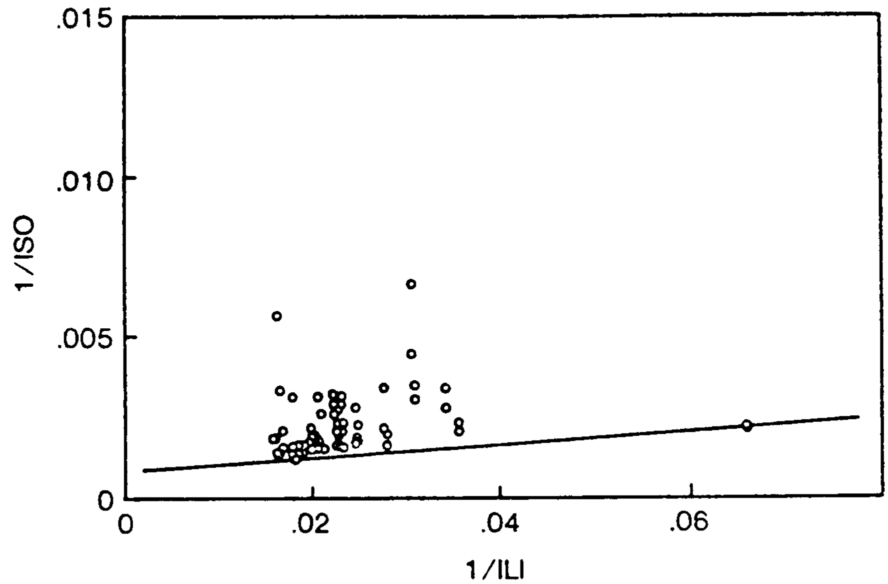

Figure 6. Double reciprocal plot of ILI and ISO. The straight line is drawn between two data points such that all other data have ordinate valves greater than or equal to the line. The equation for this line is the dashed boundary line in Figure 5.

Determining the boundary line for the data has presented something of a problem in the past but the use of a double reciprocal plot of the data (1/ISO vs 1/ILI) seems to take much of the guesswork out of the process. The data are plotted as in Figure 6 and a straight line drawn between two data points picked such that all other data have ordinate values greater than or equal to the line. The equation for this line is $1/ISO_{max} = a + b/ILI$, where a and b are the intercept and slope respectively of the double reciprocal line. Solving this equation for ISO_{max} which represents the maximum possible value for ISO at the measured ILI, yields the envelope shown as the dashed line in Figure 5. Three years of field data have shown that this procedure determines a true boundary line more accurately than our original method of using a least squares hyperbola determined from a number of points close to the boundary (solid line in Figure 5). Particularly, the double reciprocal method seems to be more realistic at the lowest levels of ILI encountered.

As an indicator of stress, the actual ISO for any day was calculated from the data and compared to the ISO_{max} corresponding to the ILI for that same day. The single number thus resulting from a days data was called the fractional integrated stomatal opening (FISO) and provides a measure of plant water stress. The question of how FISO, as a stress indicator, compares with the more conventional methods of estimating stress is the subject of the next section.

III. COMPARISON OF FISO WITH CONVENTIONAL STRESS INDICATORS

The most widely used measures of plant-water stress consist of various ways of assessing the water content of leaves, the energy level of the water in the leaves, or the degree of stomatal opening. Of these, water content is little used for field work anymore and we are left with the other two. The most widely used field method for assessing the energy content of leaf water is the measurement of leaf water potential with the Scholander pressure chamber. Measures of the degree of stomatal opening are now largely confined to measurement of the diffusion resistance of leaves with some type of diffusion porometer, preferably a steady state device. In this section of the paper we will compare the results from mass flow porometry to measurements of leaf water potential and diffusion resistance taken on the same crop over the same period of time as the mass flow data.

A. Leaf Water Potential

Midday (solar noon ± 1 hr) leaf water potentials taken on two plots of *Zea mays* in 1982 are shown in Figure 7. One plot was irrigated regularly in an attempt to maximize productivity. The other plot was subjected to two cycles of stress for comparison. The stress cycles were induced by withholding irrigation water. The first cycle was relieved by irrigation and precipitation on day 207, while the second stress period was allowed to progress through the rest of the season. Leaf water potentials were measured in each plot with a pressure chamber.

Leaf water potentials for the irrigated plot generally ranged between -1 and -1.4 MPa. While the values for the stressed plot overlapped that range considerably, they reached values as low as -1.8 MPa. But, there was never

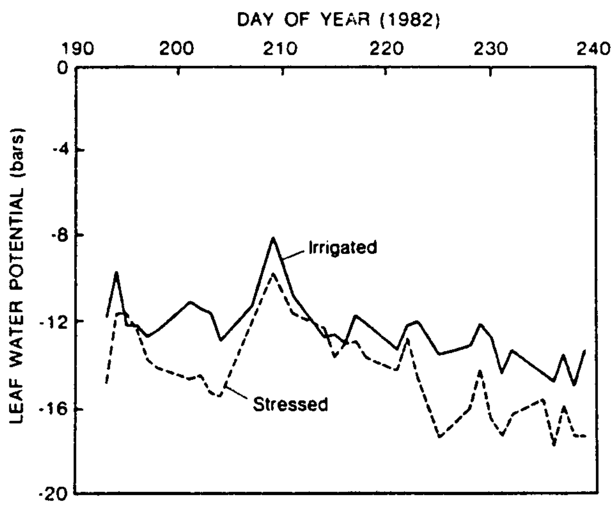

Figure 7. Midday leaf water potentials from stressed and irrigated plots.

more than a 0.4 MPa difference between them even under what appeared to be severe stress conditions, characterized by stunted vegetative growth, severe leaf curl, and discoloration.

For comparison we plotted the difference between the irrigated and stressed plot leaf water potentials, along with the fractional integrated stomatal closure (1-FISO) in Figure 8. In this instance stomatal closure was plotted so the trends would go in the same direction and make comparison easier. The first point of interest in Figure 8 is that before there was any noticeable difference in the leaf water potentials the mass flow porometers were already showing a 20% closure. Following this trend, stomatal closure reached about 50% before there was as much as a 0.2 MPa difference in leaf water potentials. After the irrigation on day 207 both

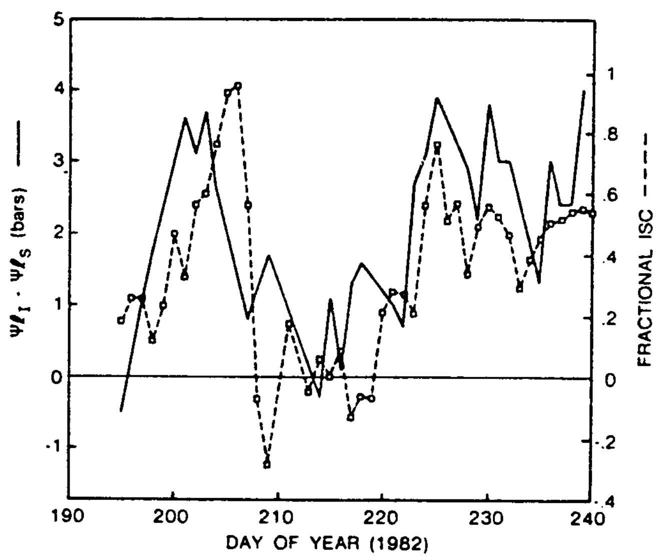

Figure 8. Comparison of FISO with the difference in midday leaf water potentials between the stressed and irrigated plots.

the potential difference and the stomatal closure decreased. Unfortunately there was a two day gap in the potential data immediately preceding the irrigation but despite the gap the trends are clear.

Also, during the first stress cycle the water potential difference appears to have reached its maximum several days before complete stomatal closure. Then after irrigation the potential difference was much slower to reach its minimum than was the stomatal closure. After the recovery period there were several days (about 215-220) when the water potential difference rose by 0.1-0.15 MPa while the stomata showed full opening. Perhaps this was an indication of some osmotic adjustment by the stressed plants. We will see other indications of adjustment when we examine the diffusion resistances for these same plants.

During the rest of the 1982 season the water potential difference and the stomatal opening seemed to track each other, with coincidental peaks and valleys. The same severity of stress was not repeated during that year, possibly because the weather was unusually cool and overcast.

B. Diffusion Resistance

Measurements of midday leaf diffusion resistance on both plots are shown with FISO in Figure 9. As with the water potential difference FISO seems already to have dropped to about 75% by the time there was any apparent difference in the diffusion resistances between the stressed and irrigated plots. Unlike the potential data, however, the diffusion resistance recovery follows very closely the recovery in FISO.

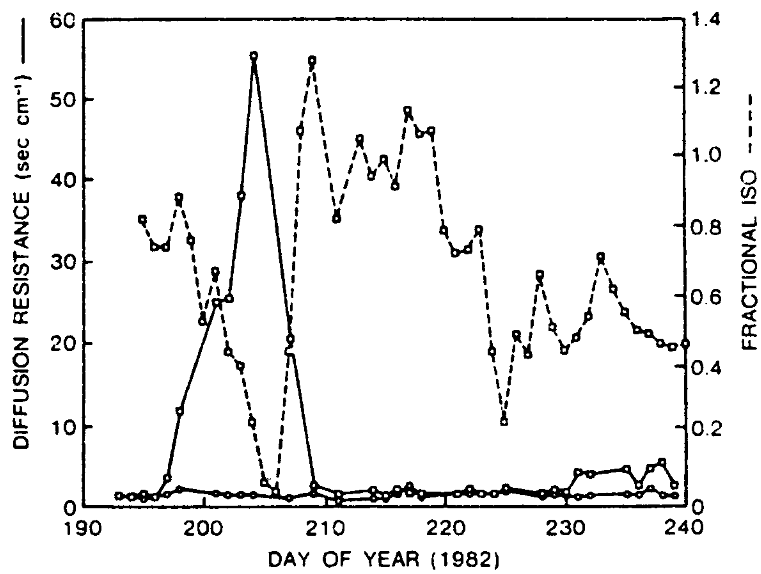

Figure 9. Comparison of FISO with midday leaf diffusion resistance determined with a steady-state porometer.

During the second stress cycle the diffusion resistance remained low and similar to the irrigated plot until 11 days after FISO reached levels that indicated moderate stress. The reasons for this apparent shift in the relationship between mass flow resistance and diffusion resistance are unclear. Perhaps the adjustment of plant water relations due to the first stress cycle involved more than simple osmotic responses. It is possible that the adjustment resulted in an increased internal mass flow resistance or an inability of the stomata to shut down as tightly at night. There are several other alternatives such as a shortening of the

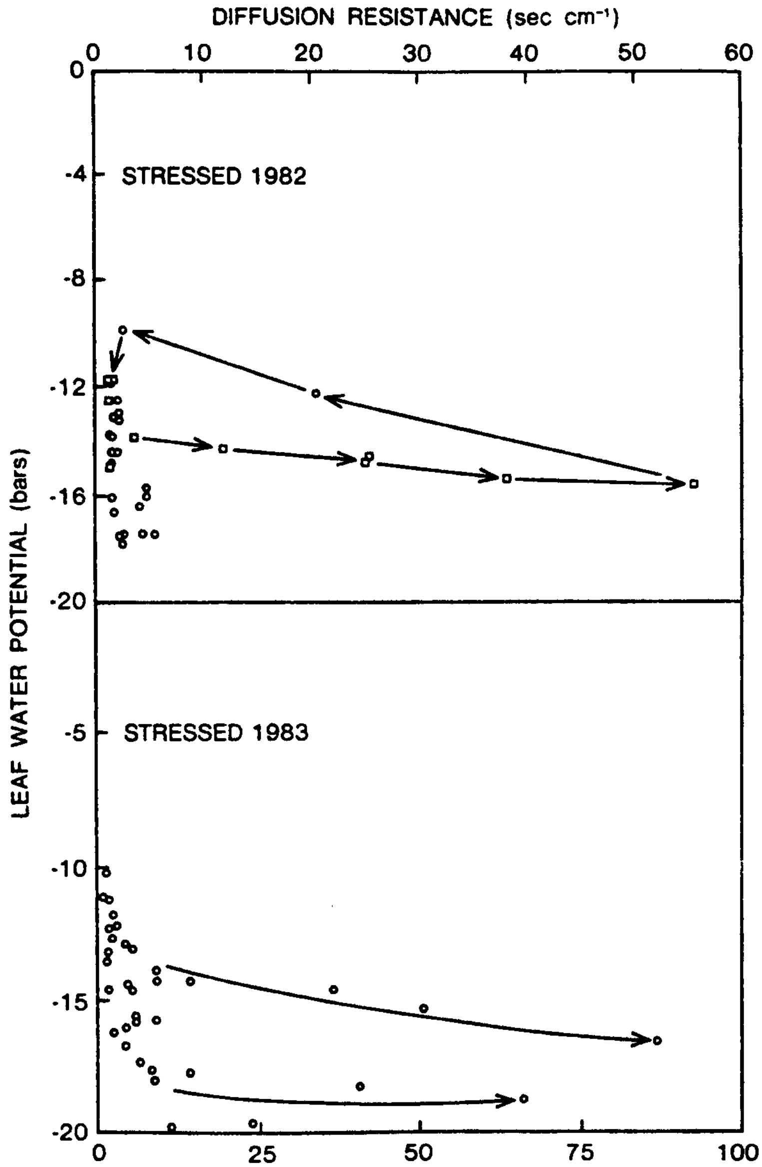

Figure 10. Relationship between midday leaf water potential and midday diffusion resistance for the stressed plots of 1982 (A) and 1983 (B). The second stress cycle in both cases shows apparent osmotic adjustment brought about by the first stress cycle.

diffusion path, a decrease in the stomatal response to light, perhaps involving an increase in the opening thereshold intensity, or a difference in response of the adaxial and abaxial stomata. Further experimentation and analysis is necessary before it will be possible to choose between these alternatives.

The expected adjustment of the relationship between diffusion resistance and leaf water potential occurred as a result of the first stress cycle. Figure 10A shows the data from 1982 when it was not possible to increase the resistance very much during the second stress cycle. Figure 10B from 1983 is included to show that when conditions are favorable for inducing stress during the latter part of the growing season the stomata are still capable of a closing response and that this closure is shifted to lower water potentials after the first stress cycle.

IV. FISO AS AN IRRIGATION CONTROL PARAMETER

Because FISO is as good an indicator of plant water stress as either diffusion resistance or leaf water potential measurements, we decided to try using it as the feedback element in an automatic irrigation control system (Fiscus et al., 1984b). The system was implemented as in Figures 11 and 12 with a small microcomputer system with data acquisition and output control capabilities. The plots where the water application was automatically controlled were irrigated with a drip (trickle) system in order to control precisely the application rates and quantities. Although there are distinct advantages to using trickle irrigation the control system should work equally well with any other type of system which could be remotely activated such as electrically operated ditch gates, center pivot systems, or automatic gated pipe. The other plots in the field (Figure 12) were furrow irrigated on an arbitrary schedule. As in previous years the data consisted of porometer pressure and PPFD measurements acquired at 10 minute intervals throughout the growing season. The integrations and calculation of FISO for each of the plots were carried out by the computer immediately after midnight each day. The flow diagram for the control program is shown in Figure 13.

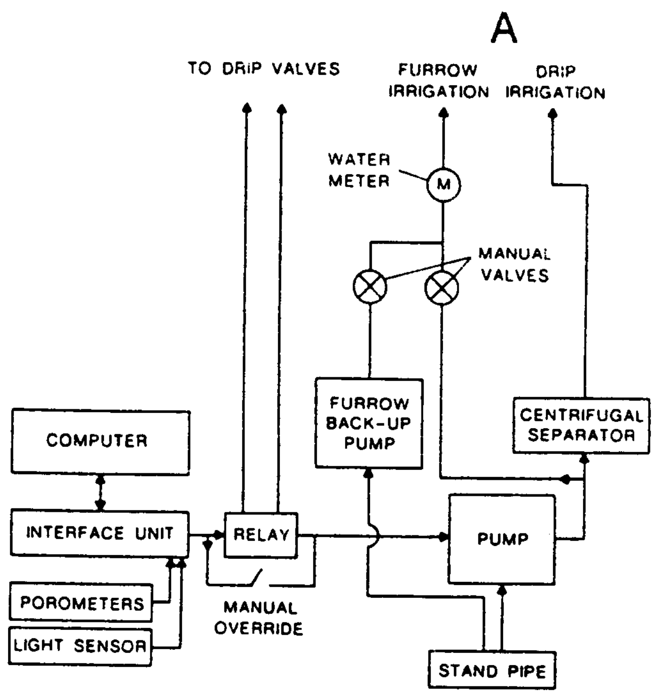

Figure 11. Diagram of computer interface and control system.

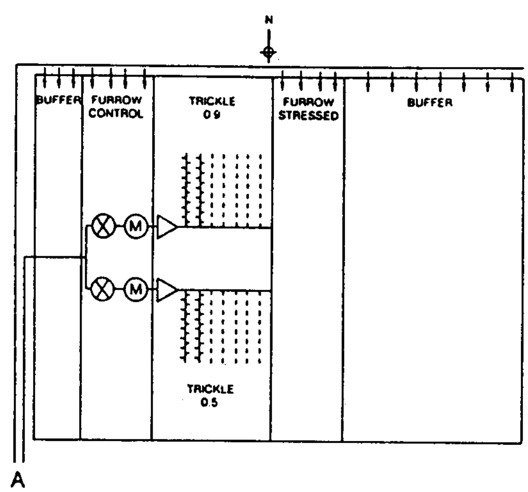

Figure 12. Layout of field plots for 1983. Ms are water meters and valves are activated by the computer.

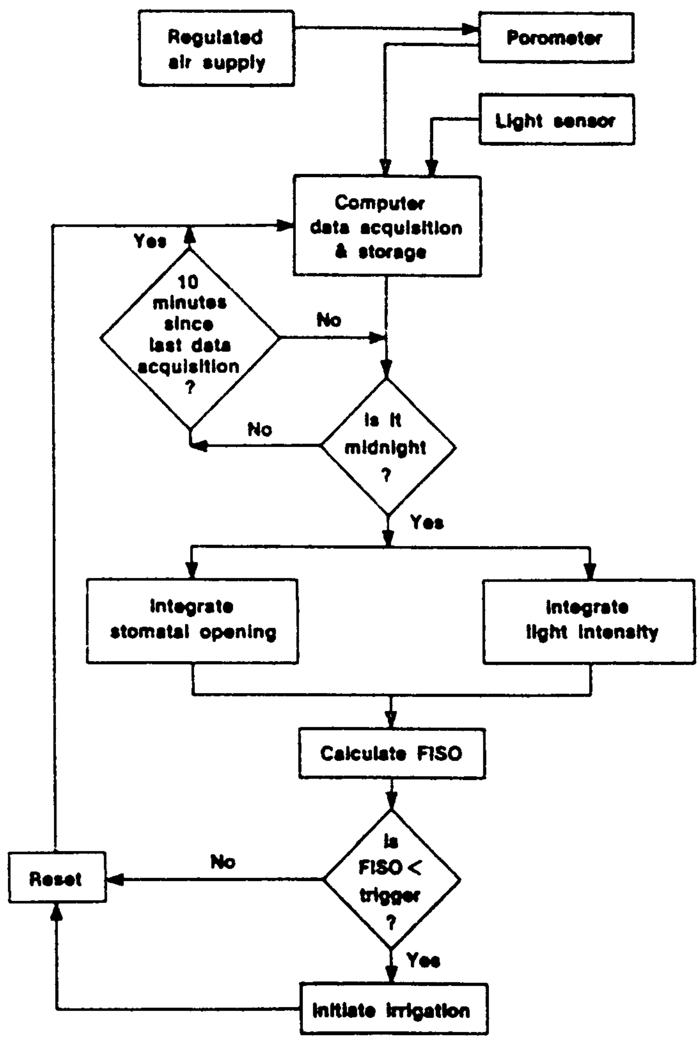

Figure 13. Flow diagram of irrigation control system program.

After calculation of FISO the computer simply checks the predetermined irrigation trigger level (that FISO below which irrigation should be initiated) for each controlled plot. If the FISO for that day was below the trigger level then the pump and appropriate valves are activated to carry out the irrigation.

In addition to the obvious advantages of such a system in terms of water conservation and irrigation efficiency it should be a very useful experimental tool in the study of stress effects in the field. Its usefulness derives from the fact that for the first time it is possible to control the level of stress in a plant population at a wide range of arbitrary levels instead of simply putting the plants through extreme drying cycles by withholding irrigation water. In effect the way the system works is simply to decrease the amplitude of the drying cycles such that variations in the degree and duration of stomatal opening are minimized. This technique should allow more detailed studies of stress adaptive processes as well as the effects of water stress on a wide range of physiological processes.

An indication of the effectiveness of the control system may be seen in Figure 14. Here, we have accumulated (1-FISO) for each day of the active growing season for each plot and expressed it as accumulated stomatal stress days (SSD). One SSD is therefore equivalent to a single 24 hr period during which the stomata do not open at all. For example, four days with FISOs equal to 0.75 would add up to 1 SSD (4 x 1-0.75). Also shown in Figure 14 are the accumulated SSDs from the

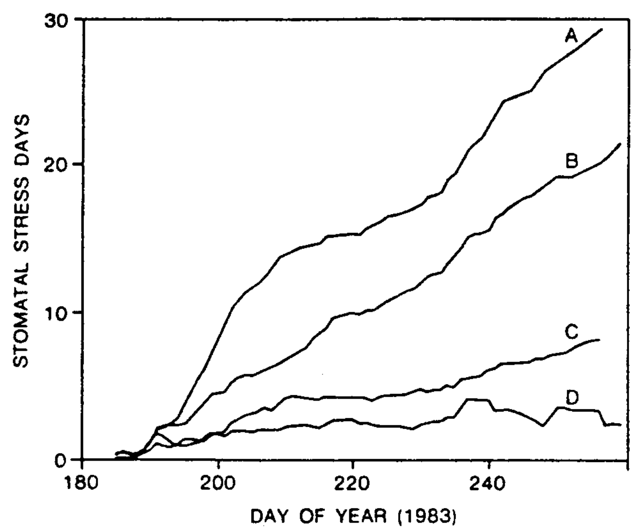

Figure 14. Accumulated stomatal stress days for the four plots of 1983. A and C are furrow irrigated stressed and control plots respectively. B and D are the drip irrigated stressed and control plots.

Finally, as a matter of practical significance in developing an irrigation control system or simply for monitoring plant stress, it is necessary to know the timing intervals required to obtain reliable data. It is also an important consideration in the development of battery operated instrumentation where power requirements and memory costs may limit the feasibility of such systems. For this purpose we analyzed the data from our 1983 field plots at different time intervals. Figure 15 shows how the scatter in the data increases as the time interval between readings is extended from 10 to 120 minutes. Figure 16 shows the r^2 values for both stressed and irrigated plots as functions of the timing interval. In both cases the r^2 declined relatively slowly up until the 60 minute interval after which the rate of decline accelerated indicating decreasing reliability of the average FISO for intervals longer than 60 minutes.

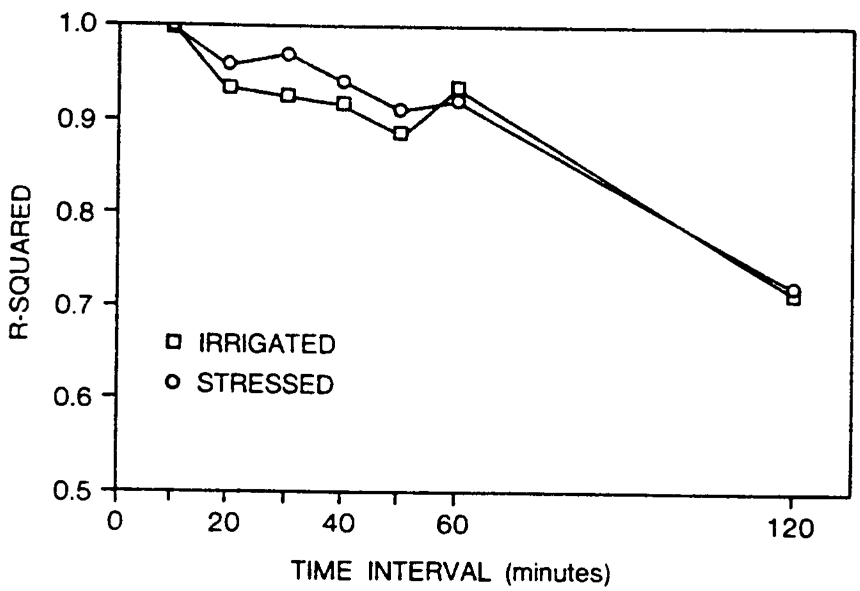

Figure 16. Decreasing data reliability with increasing sampling intervals for the stressed and irrigated plots of 1983.

The mean FISOs for 1983 are shown in Figure 17. Clearly, it makes little difference in this instance if the data acquisition interval is increased from 10 to 60 minutes. Beyond 60 minutes, however, the mean FISO shows a significant decline.

Although the mean FISOs for the whole season appear relatively insensitive to the timing interval up to 60 minutes the degree and nature of the increased data scatter dictate caution when using the system for irrigation control, especially if the objective is to minimize water use and irrigation costs. This fact is illustrated by Figure 18 which shows the amount of irrigation water which would have been applied based on the FISOs calculated at different timing intervals. Maximum conservation cannot be achieved in the well irrigated plot with timing intervals greater than about 40 minutes. In fact, if the interval had been extended to 2 hours the system would have applied more than twice the amount of water necessary to achieve good production. The reason for this apparently is that the increased data scatter resulting from the longer timing intervals increases the probability that the measured FISO will fall below the trigger point.

The conclusion to be drawn from the timing interval data is that the interval used must be determined to a very large extent by what objective is desired. If one wishes to study transient stomatal responses then readings must be taken many times per minute. If monitoring accumulated stress is all that is desired then reasonable accuracy may be achieved with intervals as great as 60 minutes. However, good irrigation control requires intervals of 40 minutes or less.

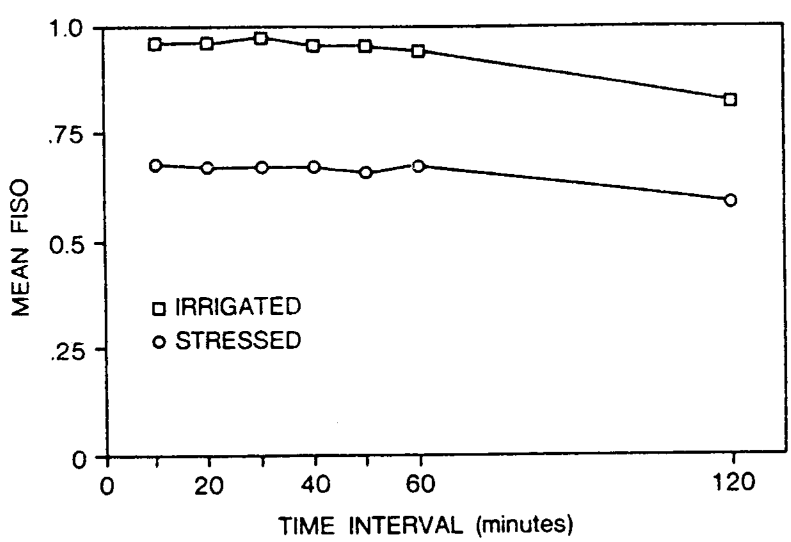

Figure 17. Mean seasonal FISOs for 1983.

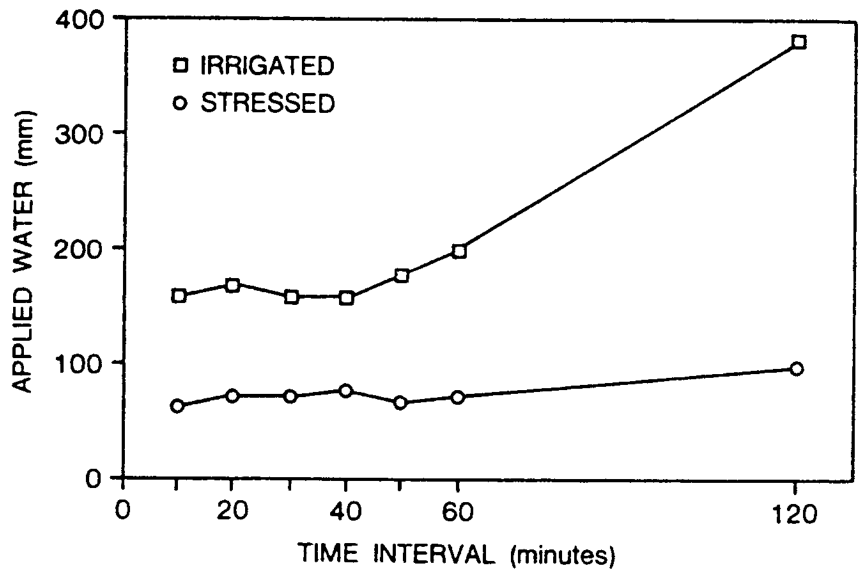

Figure 18. Effect of sampling interval on the quantity of water which would have been applied by the automatic irrigation control system. Grain yields on the two plots were 146 bushels/acre (irrigated) and 110 bushels/acre (stressed).

V. SUMMARY

In summary, the mass flow porometer is a useful technique for the detection and monitoring of water stress in the field. It compares favorably with the more traditional measures of stress such as leaf water potential and stomatal diffusion resistance. The instruments described here are well suited for continuous monitoring of stomatal aperture and therefore can be used as the feedback elements in a totally automated irrigation control system. Such a control system allows, for the first time, the growth of experimental field plants under controlled steady levels of water stress.

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APPENDIX

RSC: Relative Stomatal Closure = (P/Pmax)100, where P is the measured pressure at the lower surface of the leaf, Pmax is the maximum pressure observed during a 24 hr period indicating the greatest closure during that period. See figures 3 and 4.

RSO: Relative Stomatal Opening = 100 - RSC

ILI: Integrated Light Intensity = $\int_{0}^{24\ hr} \mathrm{PPFD}$

ISO: Integrated Stomatal Opening = $\int_{0}^{24\ hr} \mathrm{RSO - RSOref}$

RSOref: RSO reference or baseline = mean of RSO between midnight and dawn.

ISOmax: Maximum possible ISO for any ILI. This is the boundary line.

FISO: Fractional Integrated Stomatal Opening = ISO/ISOmax

FISC: Fractional Integrated Stomatal Closure = 1 - FISO

SSD: Stomatal Stress Day; equivalent to one day when FISC = 1. Fractions of FISC may be accumulted over time and the sum is an indicator of accumluted stress.