

Methods of Calculating Carbon Dioxide Exchange Rates for Maize and Soybean Using a Portable Field Chamber*

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Abstract

Accurate measurements of carbon dioxide exchange rates (F) and total evaporation (E) are essential for the evaluation of crop management practices and for the development of crop growth models. The objective of this work was to evaluate different methods of calculating the CO_2 depletion rate, *i.e.* change in CO_2 concentration (mass basis) per unit time, using a closed chamber on field plots of maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.). The three methods investigated were mean rate (MR), concentration regression (CR), and rate regression (RR) for data sets from irrigated and non-irrigated maize and soybean. The MR method was limited because of its primary dependence on the endpoint values if the measurements were equally spaced. Both CR and RR methods have implied assumptions regarding linearity of CO_2 concentration decrease. If the data are significantly curvilinear, then the RR method may be used; however, caution is needed because of the sensitivity of the RR method to the endpoints of the calculation window. Analysis of the starting time in the calculations indicated calculations should start at chamber closure after correcting for appropriate lag times. Calculating F by the RR method can typically make as much as a -27 to $+67\%$ difference in F calculated by the CR method, depending on whether the CO_2 depletion is curvilinear and the noise at the endpoints of the calculation interval. With most measurements, the CR method is recommended with automatic data systems because of its relative insensitivity to intermittent noise and conservative nature.

Precise estimates of instantaneous growth and net assimilation rates are essential for the evaluation of management practices on crop growth and development of crop growth models. These estimates, based on photosynthetic activity measured on field canopies, are usually developed using portable chambers to measure both photosynthesis and transpiration (Musgrave and Moss 1961, Puckridge 1969, Stiles and Leafe 1969, Peters *et al.* 1974, Christy and Porter 1982, Daley

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et al. 1984, Garrity *et al.* 1984, Meyer *et al.* 1987). Most workers assume a linear decrease in CO₂ concentration and either measure the time for a prescribed decrease in CO₂ or measure the decrease in CO₂ over a prescribed time. The principles involved in this approach were reviewed by Jarvis *et al.* (1971) and Lake (1972).

Field enclosures can be classified into three categories (Larcher 1969): (1) closed systems with no adjustments to CO₂ or water vapor, (2) open systems with continuous air flow through the chamber, and (3) semiclosed systems with CO₂ injected to maintain constant concentration around the plant. The main disadvantage of any chamber system is the inability to reproduce natural air movements present in a field canopy and the rapid changing conditions of leaf temperature, ambient humidity, and CO₂ concentrations during the period of measurement (Bosian 1965a, b, Jarvis 1970). In this study we are concerned with a closed chamber system with no control over water vapor increase or CO₂ decrease and the internal microclimate. Lange (1962) discussed the "Klapp-Küvette" which was the first practical chamber of this kind that was a tiltable trap-type chamber used in combination with an infrared gas analyzer (IRGA) and sequencing valves. The chamber was closed and the air was circulated through the IRGA for CO₂ analysis. The effect of the presence of the chamber on the calculations of carbon dioxide exchange rate (F) and evapotranspiration (E) measurements has not been fully explored. Previous work on small single leaf chambers has shown that CO₂ and H₂O exchange rates can be determined with sufficient accuracy to provide useful information on unit leaf area basis (McPherson *et al.* 1983, Schulze *et al.* 1982). However, there is little information using large chamber for canopy CO₂ and H₂O exchange rates on unit land area basis.

Previous experience using a large closed chamber indicated differences in CO₂ depletion rate between maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr). CO₂ depletion as a function of time is usually linear for maize and soybean but occasionally follows a curvilinear trend when soybeans are measured. This curvilinear decrease could be interpreted as a more pronounced effect of the altered microclimate within the chamber on soybeans. Later work has shown some of the curvilinearity was due to CO₂ depletion within soybean canopies with high leaf area densities. The effect of the "within canopy" depletion on calculated F is exaggerated on calm days. An additional mixing time was required to alleviate this effect. Even after allowing for additional mixing, the decrease in CO₂ was slightly curvilinear, suggesting the artificial environment within the chamber affects F . Since the same rate estimation method was commonly used for both maize and soybean, there was concern the accuracy of these assumptions may change depending on the crop being measured. Concern also exists on the accuracy of calculation methods used to account for the possible non-linear decrease in CO₂. Therefore, the objective of this work was to determine if there are differences in methods of calculating CO₂ depletion rate within a closed chamber on maize and soybean canopies. In addition, the effect of altering the starting time of the calculation window was examined as it relates to both the crop being measured and the method of CO₂ depletion rate estimation.

METHODS

Measurement principles: The principles of gas exchange measurements and calculations generally apply equally well for a CO₂ concentration decrease (F) or a H₂O vapor increase (E). For simplicity, the following discussion will pertain to CO₂ depletion rate within the chamber that can be estimated by one of the following methods: (1) Mean Rate (MR) which calculates the mean decrease in CO₂ per unit time across the measurement period, (2) Concentration Regression (CR) which cal-

calculates the slope of the least squares line relating CO₂ concentration to time, or (3) Rate Regression (RR) which determines the intercept of a least squares line between point estimates of decrease in CO₂ per unit time and the midpoints of the associated time intervals*. The regression of the individual rates is used to estimate the intercept as the initial rate. These methods were used in a selected calculation window within the total data set collected at 2 s intervals. Use of either the MR or CR methods assumes a linear decrease in CO₂ concentration with time in the calculation window, while use of RR implies a quadratic relation between CO₂ and time. Jarvis *et al.* (1971) expressed F [g(CO₂) m⁻² h⁻¹] as:

$$F = (\Delta C_m / \Delta t) \cdot (V_c / A) \quad (1)$$

where $\Delta C_m / \Delta t$ = change in CO₂ concentration (mass basis) per unit time [g(CO₂) m⁻³ h⁻¹], V_c = volume of air within the chamber [m³], and A = soil area under the chamber [m²].

In Eq. (1), $\Delta C_m / \Delta t$ is the CO₂ depletion rate occurring within the chamber. Since F is generally expressed as a mass flux of CO₂ and measured on a volume basis, it is necessary to know the air temperature and barometric pressure and to invoke the ideal gas law. If the chamber is well stirred, then CO₂ concentration in a continuously drawn sample can be assumed to be equal to the ambient CO₂ concentration. Mass concentration of CO₂ within the chamber at a given point in time (t) is:

$$C_{m_t} = \frac{C_{v_t} \cdot M_r \cdot T_0 \cdot P_i}{V \cdot T_t \cdot P_0} \quad (2)$$

where C_{m_t} = mass concentration of CO₂ within the chamber at time t_t [g m⁻³]; C_{v_t} = volume concentration of CO₂ within the chamber at t_t [μmol mol⁻¹]; M_r = relative mass of CO₂ (44 g mol⁻¹); V = molar volume of CO₂ (0.022414 m³ mol⁻¹) at 1013.25 Pa; T_t = temperature at t_t [K], P_i = barometric pressure at t_t [Pa], T_0 = standard temperature (273.16 K), and P_0 = standard barometric pressure [1013.25 Pa].

Mean Rate method estimates $\Delta C_m / \Delta t$ as the average of the individual $\Delta C_{m_i} / \Delta t_i$ over the calculation window:

$$\Delta C_m / \Delta t = \left[\sum_{i=1}^{n-1} \frac{C_{m_{i+1}} - C_{m_i}}{t_{i+1} - t_i} \right] (n-1)^{-1} \quad (3)$$

where n = number of data points in the calculation window. A serious problem with this estimate occurs when measurements are equally spaced. Expanding Eq. (3) with $t_{i+1} - t_i = K$ (K = an arbitrary constant) indicates that all terms but the first and last will cancel. Thus, $\Delta C_m / \Delta t$ becomes:

$$\Delta C_m / \Delta t = \frac{C_{m_n} - C_{m_1}}{K(n-1)} \quad (4)$$

* This method is in the Instruction Manual for the *Li-Cor LI-6000* Portable Photosynthesis System.

This estimate of $\Delta C_m/\Delta t$ can be seriously biased if either or both the endpoint measurements of C_m are erratic or if CO₂ depletion is not linear. Because many chamber systems usually include computer-controlled data acquisition systems, with equidistant measurement times, and because the observed CO₂ depletion rate may be curvilinear, the use of the MR method is not recommended. Therefore, the remainder of this paper will compare the CR and RR methods.

Concentration Regression method: In the CR method used by McPherson *et al.* (1983) and Meyer *et al.* (1987), $\Delta C_m/\Delta t$ is estimated as the slope of the least squares line relating the C_{m_i} to time (t_i) using the equation:

$$C_{m_i} = B_0 + B_1[t_i] \quad (5)$$

where B_0 , B_1 = intercept and slope estimated by least squares regression. B_1 is an unbiased estimate of $\Delta C_m/\Delta t$ provided the C_{m_i} decreases linearly over the calculation window.

Enclosing the plant canopy in an assimilation chamber without climate control inevitably creates an artificial environment. The basic premise in this work is that the period of data collection should be as short as possible to minimize the theoretical considerations that could result in a non-linear decrease in CO₂ (Jarvis 1970).

Rate Regression method: The RR method was selected to account for non-linear changes in gas concentration that may result from the presence of the chamber or any other factor that would decrease F or E . The method puts more emphasis on the data collected at the start of the calculation window by extrapolating the rate to time zero. If CO₂ depletion corresponds to a quadratic function of time, $\Delta C_m/\Delta t$ can be estimated by fitting a least squares regression to the $n - 1$ values of $\Delta C_{m_i}/\Delta t_i$ where

$$\Delta C_{m_i}/\Delta t_i = \frac{C_{m_{i+1}} - C_{m_i}}{t_{i+1} - t_i} \quad (6)$$

The independent variable in this case is the midpoint of each 2 s interval (t_{mid_i}), thus

$$\Delta C_{m_i}/\Delta t_i = B_0 + B_1(t_{mid_i})/2 \quad (7)$$

where

$$t_{mid_i} = (t_i + t_{i+1})/2.$$

If the chamber exerts no effect on the linear decrease of CO₂ concentration with time, B_1 in Eq. (7) will have a true value of zero. However, if the chamber's presence causes the relationship to become non-linear (optimally quadratic), $B_1(t_{mid_i})$ is an estimate of the increasing magnitude of the chamber effect with time. Therefore, if t_{mid_i} is set to zero, the intercept, B_0 , is an estimate of $\Delta C_m/\Delta t$ prior to the onset of the chamber effect. Using the RR method, B_0 from Eq. (7) is substituted for $\Delta C_m/\Delta t$ in Eq. (1).

The RR method may not be the best approach when the relationship between $C_{m,t}$ and time is nearly linear because of undue emphasis on the endpoints. Using the least squares, B_0 , from Eq. (7) is estimated as:

$$B_0 = \Delta C_m / \Delta t - B_1(t_{mid,t}) \quad (8)$$

where $\Delta C_m / \Delta t$ = average of the observed rates [Equivalent to Eq. (4)].

If the response is linear, implying the true value of B_1 is zero, estimates of B_0 and the $\Delta C_m / \Delta t$ estimates are biased by $-B_1(t_{mid,t})$. Erratic $C_{m,t}$ at the ends of the calculation window can greatly affect these estimates, as will be illustrated later. For example, an estimate of $\Delta C_m / \Delta t$ decreased 20% in an independent data set when erratic values of C_m near the beginning of the estimation period were removed. This is due to the dependence of B_0 in Eq. (8) on the estimate of $\Delta C_m / \Delta t$ given by Eq. (4) which is dependent solely on the endpoint values when the measurements are equally spaced.

CO₂ depletion was considered non-linear if B_1 in Eq. (7) significantly differed from zero at the 5% level. A *T*-test with $n-2$, in this case 13, degrees of freedom, for 15 values of $C_{m,t}$ collected over 30 s was used to test the significance of B_1 :

$$T = B_1 / S(B_1) \quad (9)$$

where $S(B_1)$ = the standard error associated with B_1 in Eq. (7).

Field measurement system: Field data from 1985–1987 were used to compare CR and RR estimates collected on maize and soybean grown on a Sioux sandy loam soil (family sandy skeletal mixed, subgroup Udorthentic Haploborol) that required irrigation for optimum production. Cultivars of Pioneer 3906 maize and Evans (maturity group 0) soybean were planted using conventional methods with a row spacing of 0.76 m. One-half of the plots were sprinkler-irrigated when the soil matric potential at 0.30 m reached -30 kPa.

The chamber, mounted on the front of a tractor, was operated in the same manner described by Reicosky and Peters (1977). Approximately 2 m of two rows was removed from the end of each plot, enabling placement of the chamber near the plot center to minimize edge effects. Due to differences in crop height, chamber volume was 3.25 m³ for soybean and 8.15 m³ for maize. Soil area covered by the chamber was 2.67 m² for both crops. All data presented here represent net CO₂ and H₂O vapor exchange, including soil respiration and evaporation. A BINOS-model 4b.2 (*Leybold Heraeus*) IRGA was used to measure CO₂ and H₂O concentration in the differential mode (range of $\pm 50 \mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$ and $\pm 10\,000 \mu\text{mol}(\text{H}_2\text{O}) \text{ mol}^{-1}$). The air within the chamber was mixed with 4 fans (0.22 m³ s⁻¹ each) and the gas sample was pumped to the IRGA through a 6.35 mm ID polypropylene tube at 560 cm³ s⁻¹. A portion of the gas was subsampled at 41.7 cm³ s⁻¹ for analysis. Another portion of the sample was drawn off to a 0.065 m³ reference tank at 41.7 cm³ s⁻¹ to isolate and buffer fluctuations in the reference gas. The CO₂ and H₂O vapor

concentrations in ambient air were used to allow the reference gases to follow diurnal changes in concentration that were as large as 30 and 5 000 $\mu\text{mol mol}^{-1}$ for CO₂ and H₂O, respectively. During the 80 s data collection period, analog output from the two channels of the *IRGA* along with other microclimate data was recorded every 2 s *via* a computer-controlled data acquisition system. All of the equipment was contained in an instrument shelter mounted on the back of the tractor. The *IRGA* output, air temperature, and solar irradiance were also recorded on a strip chart to provide immediate visual evidence of any erratic behavior in the change in CO₂ and H₂O vapor concentrations. Within the 80 s data collection period, a 30 s calculation window or interval was initially selected based on experience to calculate F and E . Several other calculation windows were tried; but experience showed 15 measurements of C_m , corresponding to a 30 s calculation window, were best to routinely calculate $\Delta C_m/\Delta t$ using the CR method. This represented a compromise between the minimum number of data points required to give a calculated value with acceptable variability (r^2 consistently 0.98 or higher) and a small enough window so that the effect of the altered environment within the chamber is minimized. The 14 corresponding point estimates of $\Delta C_{m_i}/\Delta t_i$ were used in the RR method. The calculation window normally began 16 s after chamber closure to ensure adequate lag time for the gas sample to reach the *IRGA*. This lag was determined by measuring the time required for a pulse of CO₂ injected in the empty chamber to be registered by the *IRGA*. However, as was determined subsequently, an additional lag of 10 s had to be added to allow adequate mixing with a dense soybean canopy in the chamber.

Overall system accuracy: Using the sensitive differential *IRGA* for both water vapor and CO₂ and an automatic data acquisition system, operating off a portable generator required the evaluation of potential associated electrical errors. The magnitude of the error associated with the method of measurement, the calculation, and the sensitivity of the equipment was determined using a 2 s sampling interval and data collection period of 5 min. The fluctuation in the apparent gas concentration as a result of using the "floating" reference gas for water vapor and CO₂ was evaluated in addition to those possibly caused by the electrical noise associated with the portable generator. With the large chamber in the up position and using normal gas flow procedures, the fluctuations in the incrementally calculated F using the CR method and a 30 s calculation window, were relatively large due to fluctuations in the sample gas. This was confirmed by operating the chamber in the down position over stainless steel sheets sealed to prevent CO₂ from soil respiration entering the chamber and mixing of the air in the chamber with ambient gas outside the chamber. Several data collection periods were used to evaluate the fluctuations in the reference and sample gas to establish the overall accuracy of the system.

RESULTS

Overall System Accuracy

The rates of change of the differential CO_2 concentration as a function of time with the chamber over the stainless steel sheets are summarized in Fig. 1. The differential

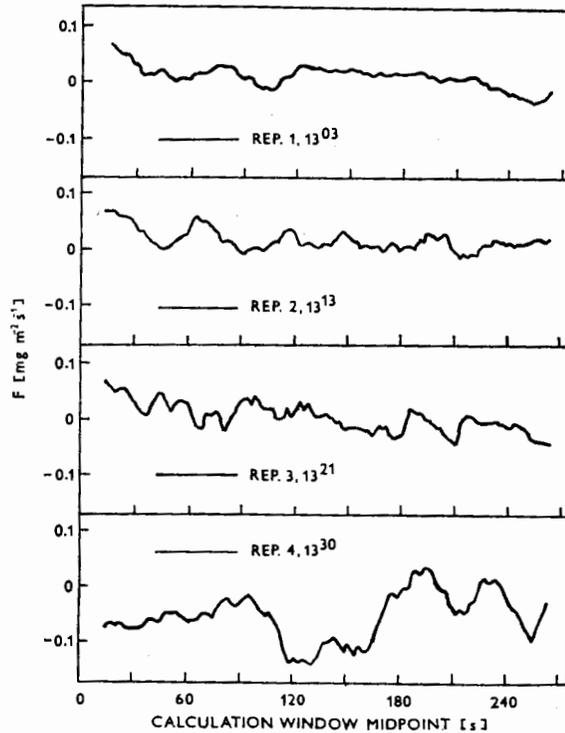


Fig. 1. Summary of four data sets of the change in the calculated F measured over stainless steel sheets for 5 min using the CR method and 15 data points.

concentration ranged from $15\text{--}20\ \mu\text{mol mol}^{-1}$ on the four different runs, reflecting a difference between the reference and ambient, but was fairly constant within a given data collection period. The calculated F within any one of the 30 s windows of the data sets for the 5 min period changed a maximum of $0.14\ \text{mg m}^{-2}\ \text{s}^{-1}$ and for three of the four data sets was less than $0.06\ \text{mg m}^{-2}\ \text{s}^{-1}$, suggesting little variation in the differential CO_2 concentration as a function of time with the chamber in the down position. The mean F values for each run were near zero, and the standard deviation within a data set ranged from 0.017 to $0.043\ \text{mg m}^{-2}\ \text{s}^{-1}$. The variations in F from the range of standard deviation, using the CR method, suggest a typical range for

the F fluctuations from -0.06 to $+0.06$ $\text{mg}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$. Similar data for the differential CO₂ concentration as a function of time with the chamber in the up position showed F fluctuations as large as $0.6 \text{ mg m}^{-2} \text{ s}^{-1}$ during the 5 min data collection period due to extraneous sources of CO₂ (data not shown). These preliminary results give estimates of the system accuracy and give some confidence that the errors in the measurements with the portable chamber using a portable generator as a power source were not significant, provided extraneous sources of CO₂ are not a problem. The small measurement error when the chamber was used in the down position suggested use of a floating reference gas with the IRGA in the differential mode for the measurement and calculations of F was reasonable.

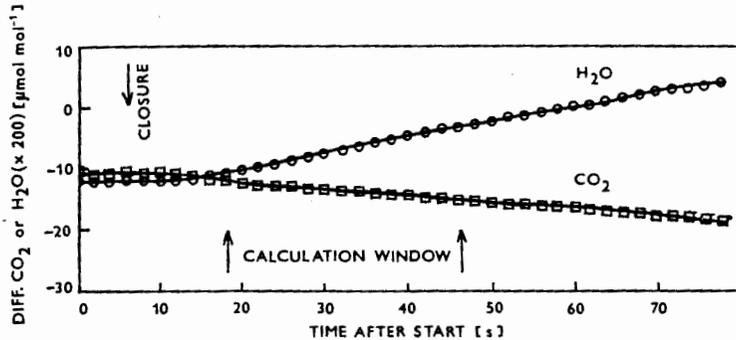


Fig. 2. Typical set of CO₂ and H₂O concentrations collected over irrigated maize NTCI with chamber closure, lag time, and the first 30 s calculation window identified. 1 September 1987, time 11¹¹. $E = 0.41 \text{ mm h}^{-1}$, $R^2 = 0.996$, $F = 0.61 \text{ mg m}^{-2} \text{ s}^{-1}$, $R^2 = 0.996$.

Maize Measurements

An example of a typical data set collected over a maize canopy and the selected calculation window for F and E shows a fairly linear decrease in CO₂ and a linear increase in H₂O (Fig. 2). The timing of chamber closure, gas sample lag time, and the calculation window within the data collection period are illustrated. This format was used in all calculations until it was determined the higher leaf area density in soybean canopy required an additional mixing lag.

An example of the calculation of F with a linear decrease in CO₂ for maize plants is illustrated in Fig. 3. Calculation windows of 10, 20, and 30 s (5, 10, and 15 data points) followed the 16 s lag from closure to calculate F by CR and RR methods. The calculations were continued on an incremental basis by moving the calculation window through the data, sequentially dropping the first data point and picking up the next data point to give F plotted as a function of time through the remainder of the data. There was little change in F as a function of time in the measurement interval using the CR method with a 30 s calculation window. F calculated using 10

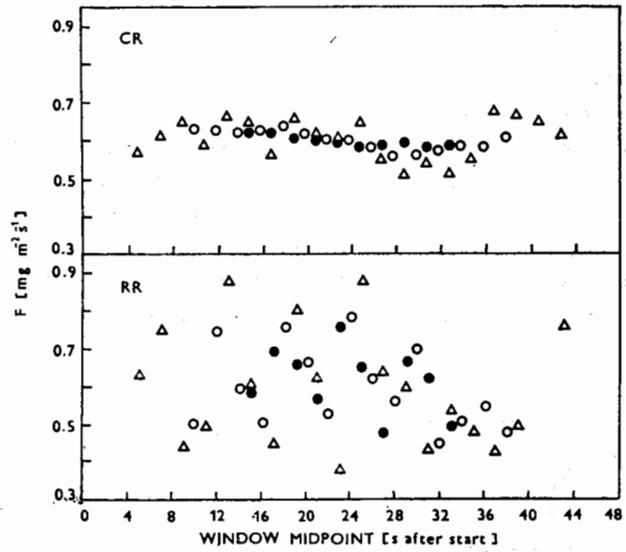


Fig. 3. F calculated by the CR and RR methods on the raw data for maize NTCI (Fig. 2) using 5 (Δ), 10 (\circ), and 15 (\bullet) data points in the calculation window. 1 September 1987, time 11¹¹.

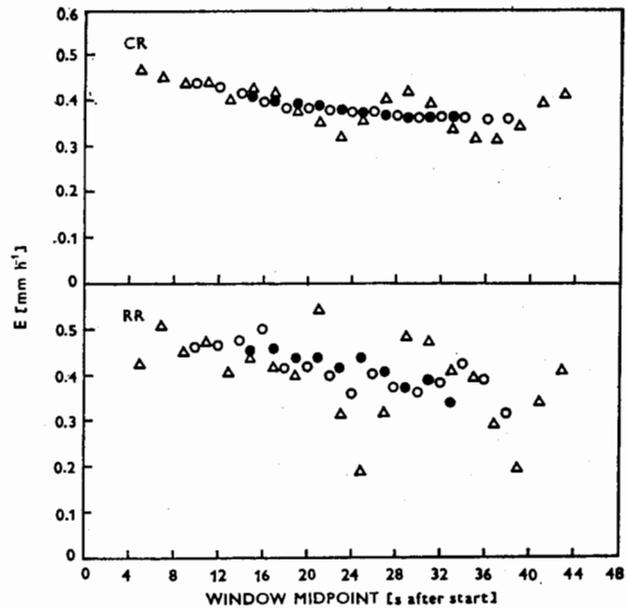


Fig. 4. E calculated by the CR and RR methods on the raw data for maize (Fig. 2) using 5, 10, and 15 data points in the calculation window. 1 September 1987, time 11¹¹.

and 20 s intervals showed more scatter as a result of fewer values in the regression. However, using the RR method the values of F were occasionally higher and more erratic than those calculated by the CR method. Even though there were no obvious noise points in this data set, the sensitivity of the RR method to the endpoint within the calculation window resulted in more fluctuations in F as the calculation window moved through the data.

Total evaporation (E)* calculated using the raw water vapor data (Fig. 2) and calculated in a similar manner as F (Fig. 3) is summarized in Fig. 4. As in the case of F , E showed more erratic behavior when calculated by the RR method compared to the CR method. The shorter calculation windows showed more erratic behavior in both the CR and RR methods. Both methods showed a tendency for the E to decrease with time as might be expected with the increase in water vapor concentration within the chamber (Daley *et al.* 1984).

Soybean Measurements

During the three growing seasons used in this study, numerous individual F measurements were made on various soybean and maize plots. Visual inspection of the strip chart trace of the individual data sets of CO₂ showed that about 50 % of the soybean data had a slight curvilinear change in the differential CO₂ concentration with time while most of the measurements on maize plots showed a linear change. The curvilinear changes in CO₂ on the soybean were generally distributed equally between

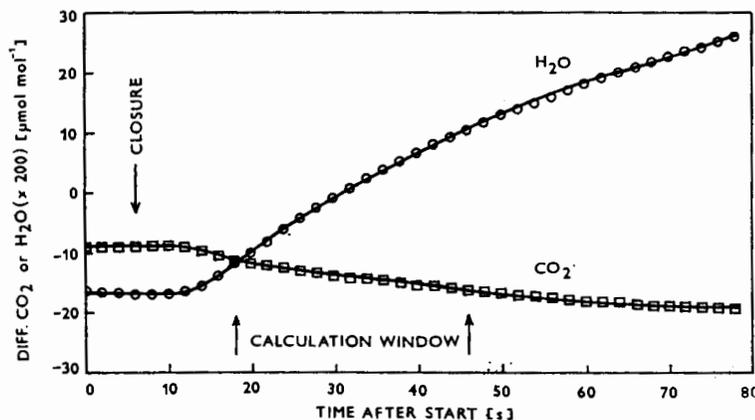


Fig. 5. Typical set of CO₂ and H₂O concentrations collected over irrigated soybean CTSI with chamber closure, lag time, and the first 30 s calculation window identified. 30 June 1987, time 10⁴³. $E = 0.44 \text{ mm h}^{-1}$, $R^2 = 0.995$, $F = 0.35 \text{ mg m}^{-2} \text{ s}^{-1}$, $R^2 = 0.993$.

* The values of E are expressed in the traditional micrometeorological way as $[\text{mm h}^{-1}]$, even if this expression does not agree with the SI system of units.

irrigated and non-irrigated treatments, with a slight tendency to be more dramatic on those plots that showed water stress.

For a typical curvilinear data set of soybean (Fig. 5) F was calculated the same as for maize (Fig. 6). Using the CR method showed a gradual decrease with time in the data collection period for all three calculation windows. The scatter with 5 data points

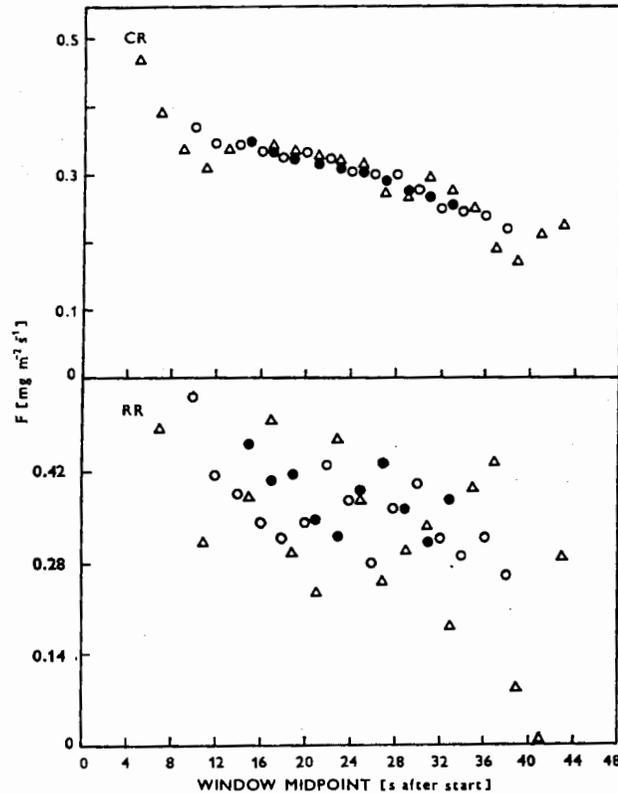


Fig. 6. F calculated by the CR and RR methods on the raw data for soybean CTSI (Fig. 5) using 5 (Δ), 10 (\circ), and 15 (\bullet) data points in the calculating window. 30 June 1987, time $10^{4.3}$.

was only slightly larger than for 10 and 15 data points. The RR method showed a more erratic F for all calculation windows that also decreased with time even after correcting for an additional mixing lag. Preliminary results based on the standard 16 s lag time required to get the gas sample from the chamber to the IRGA showed a dramatic decrease in F in the first 10 s after closure. This brief period of rapid decrease was later determined to be a result of CO_2 depletion within the dense canopy of soybean and resulted in an apparent exaggerated depletion rate. The higher leaf area index and leaf area density for soybean during periods of low wind resulted

in this depletion of CO₂ that, when mixed with the ambient air, gave an exaggerated decrease in CO₂. An additional 10 s lag time was added to account for this "dilution effect" with F values calculated using both CR and RR methods (Fig. 6). The fluctuations in F were more dramatic using the RR method because of the influence of the end points and the curvature early in the calculation window. These results showed the sensitivity of the two methods on the magnitude of F using a 15-data point interval. The differences in F for the two methods are substantially larger than the total system error (Fig. 1) and suggest judgment in determining which method should be used.

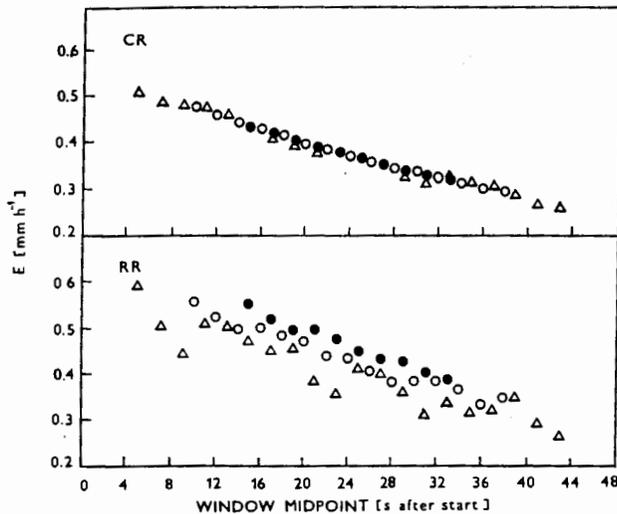


Fig. 7. E calculated by the CR and RR methods on the raw data for soybean (Fig. 5) using 5, 10, and 15 data points in the calculation window. 30 June 1987, time 10^{43} .

The cause of the differences in CO₂ depletion rate for maize and soybean may be related to species effects (C_3 vs. C_4) or to soybean sensitivity to water stress and higher leaf area density. Whatever the causative mechanism may be, it is most likely related to differing response to the presence of the chamber. A testable criteria is needed to determine objectively whether CR [Eq. (5)] or RR [Eq. (7)] is appropriate. The criteria used was a T -test for the significance of B_1 in Eq. (7). If B_1 was not significantly different from zero at the 5% level, CR should be used to estimate $\Delta C_m/\Delta t$. If B_1 is significant, $\Delta C_m/\Delta t$ should be estimated using RR. This test was programmed into the chamber's computer-controlled data acquisition system to provide a selection of the appropriate method. In Fig. 4, the T -test with $T = 0.05$ did not show the slope in the RR method significantly different from 0. In practice, the utility of this criteria was overshadowed by other problems of the RR method.

The soybean E calculated by the CR method (Fig. 7) showed little scatter in E for the various calculation windows and a gradual decrease as a function of time similar to maize, while when calculated by the RR method it showed more scatter and a similar decrease with time. While all three calculation windows show the same trend with time, the E calculated using the RR method with 5 data points is generally lower than that with 10 or 15-data points. The E calculated using both CR and RR methods on the first 30 s window was 0.44 and 0.57 mm h⁻¹, respectively. The larger E calculated using the RR method reflects the non-linear increase in vapor density when the rate is extrapolated back to time zero.

The chamber used in this study was placed over the canopy as a unit and resulted in the initiation of mixing within the canopy when the chamber made contact with the soil. This mode of operation was slightly different than that described by Peters *et al.* (1974) and Christy and Porter (1982). Their chambers moved on a "rail system" with the ends as flexible doors rolled up similar to a window blind during chamber travel. The chamber is moved over the plots, set in position with the fans constantly mixing the air. After a signal to close the doors (*ca.* 18 s), the doors will close while the fans are mixing the air. This continuous mixing during closure results in a decrease in the effect of the CO₂ depletion within the enclosed canopy and would not cause the initial rapid draw-down in CO₂ observed with the closed portable chamber in this work.

Method Selection

If the CO₂ concentration as a function of time is truly curvilinear as a result of some "chamber effect" other than the canopy dilution effect mentioned previously, there may be need to account for this effect. In the upper portion of Fig. 6 the results using the CR method for the three time intervals (10, 20, 30 s) in the data set show a gradual decrease as the width of the calculation window increased. The rate of decrease of F as a function of time in the measurement cycle decreased as more data points were included. In choosing the "best" interval for calculation of F there appears to be some trade-off; with more data points the correlation coefficient (R^2) is generally higher but the F is generally lower. The curvature of the data results in a more dramatic change in F as a function of time and is not adequately reflected in the R^2 . The differences in F using the RR and CR methods are large enough to require objective judgment on which method should be used. The T -test is one method that would allow the data to determine whether the CR or RR method should be used. However, the selection of the critical value for the T -test is somewhat subjective. Even after correcting for the additional mixing lag, numerous observations on stressed soybean occasionally suggest the RR method may be used because of the curvilinear change in CO₂ concentration. However, caution may be needed due to the sensitivity and dependence of F on the endpoints of the calculation window. The apparent increase in leaf temperature during the measurements that are substantially higher on

the non-irrigated than on the irrigated treatments may result in a physiological response in the plants and thus a curvilinear trend in the data.

Because of the fluctuations in F using the incremental calculations for both CR and RR methods, it was decided to test the effect of noise at the endpoints of the calculation window using a mathematically "smooth" data set of CO₂ concentration as a function of time generated using a cubic polynomial. This form was selected because of the visual similarity to typical measurements but without any noise. The endpoints for the 30 s calculation window were varied by 1 $\mu\text{mol mol}^{-1}$, about a 0.3 % change in absolute CO₂ or $\approx 7\%$ change in the depletion in various combinations. In general, the percent error in F relative to F with no "endpoint" noise for the CR method was small and ranged from 4.7 % to 9.5 % when there was "endpoint" noise in opposite directions (Table 1). However, using the RR method, there was larger error that ranged from as low as -21.3 % to as much as 57.1 %. The RR method shows more sensitivity to noise at the endpoints of the calculation window that results in exaggerated values of F . The CR method is more conservative in that the effect of the error at the endpoints is smaller, resulting from apparent averaging within the CR method. The sensitivity of the RR method to the endpoints of the calculation window needs to be considered in selecting the best method for calculating canopy F .

Table 1

The effect of noise on the F [$\text{mg m}^{-2} \text{s}^{-1}$] calculated by Concentration Regression and Rate Regression for a curvilinear data set using a 30 s calculation window.

"Endpoint noise"		Concentration Regression		Rate Regression	
left	right	F	% error	F	% error
0	0	1.40	—	1.48	—
+1	0	1.47	+4.7	2.20	+57.1
0	+1	1.34	-4.7	1.83	+30.1
-1	0	1.34	-4.7	0.76	-45.6
0	-1	1.47	+4.7	1.14	-18.6
+1	-1	1.54	+9.5	1.86	+32.8
-1	+1	1.27	-9.5	1.11	-21.3

Janáč *et al.* (1971) showed several examples of the standard deviation of the photosynthetic rate calculated from hypothetical values assuming differing kinds of measurement. For the differential mode with a background of about 300 $\mu\text{mol mol}^{-1}$ CO₂, the cumulative error or the standard deviation for the calculated photosynthetic rate was $\pm 3.6\%$ and could be as large as 5.7 %. This included hypothetical considerations of the accuracy of the CO₂ analyzers on the full-scale reading, the accuracy in determining the flow rate, and the accuracy in determining the leaf area inside the

chamber. The magnitude of the error due to noisy endpoints in this work was generally larger than the theoretical estimates of Janáč *et al.* (1971).

Variation in starting time of the calculation window had little effect on maize F using the CR method. Once the sample reached the IRGA, the change in F estimates was essentially constant (Fig. 3). However, if $\Delta C_m/\Delta t$ was estimated by RR, F estimates showed more dependence on starting time. This variation is primarily a result of bias introduced into the estimate by applying RR to linear data and the dependence of this bias on the calculation window endpoints. Some of the variation may be due to minor fluctuations in the reference gas; however, the results in Fig. 1 suggest that this is small.

In principle, the calculations of the water vapor increase in the chamber to calculate E should be the same as for the CO_2 decrease. In practice, the increase in H_2O vapor was more curvilinear and shorter and showed a slightly greater tendency to plateau for both maize and soybean as saturation was approached (see Figs. 2 and 5). Similar results have been noted by Daley *et al.* (1984) and confirm the need to keep the calculation window as narrow as practical and start immediately after the lag correction. The water vapor build-up in the chamber has a greater effect on E than does the CO_2 depletion on F . These differences were more noticeable on soybean than maize. While the absolute concentrations of the two gases are vastly different, these results require the earliest possible calculation window to minimize the effect of water vapor build-up on the measured E .

CONCLUSIONS

The decrease in CO_2 concentration within the closed chamber was essentially linear when the measurements were taken over maize. However, the decrease was occasionally curvilinear when soybean was measured, particularly when the plants were undergoing some water stress. An additional compounding factor on the soybean was the apparent dilution effect as a result of higher leaf area density of the soybean canopy that resulted in an apparent exaggerated shortterm rate of CO_2 depletion. After correcting for an additional lag time for complete mixing, there was less curvilinearity in the CO_2 data. If the decrease in CO_2 is linear, then the CR method is appropriate for estimating F . The RR should only be used when the CO_2 decreases are curvilinear throughout the entire measurement period and there is no noise at the endpoints of the calculation window. If the point estimates decrease significantly with time, the RR may be appropriate. If not, then F should be estimated using the CR method because of its conservative nature.

For both maize and soybean the calculation window of 30 s was best and should begin as soon as possible after chamber closure. Although soybean measurements are more susceptible to variation associated with the starting time, the possible chamber effects can be minimized by using the earliest calculation window and

partially corrected for by using the RR method if the raw data is very smooth. However, due caution is needed utilizing the RR method because of its sensitivity to the endpoints that might result in exaggerated values of *F* and *E* for both maize and soybean.

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