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AN I.R.G.A. SYSTEM FOR CONTINUOUS MONITORING OF CO_2 AND H_2O VAPOUR EXCHANGE IN REPLICATE PLANTS GROWING IN CONTROLLED ENVIRONMENTS

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INTRODUCTION

I.R.G.A.: description, principle and uses.

An I.R.G.A. (Infra-red Gas Analyser) determines the concentration of a gas or vapour in a gaseous diluent by measuring absorption of infra-red radiation. Many gases and vapours have suitable absorption spectra in the infra-red region, and IRGAs find applications particularly for measuring with water vapour, the oxides of carbon, nitrogen and sulphur, and many hydrocarbons. Due to the presence of many absorption bands in most spectra, the most sensitive and inteference-free analysers are based on a detector which incorporates a filter containing a pure sample of the gas to be measured, though some machines use optical filters. The gas filled filters are not easily replaced and therefore in practice this form of IRGA cannot be conveniently or quickly changed from measuring one gas to another.

In plant physiology the most commonly used forms of IRGA are those which measure carbon dioxide and water vapour which permit the determination of net CO_2 fixation and water vapour loss (usually transpirational) by plants. Net CO_2 fixation is a measure of the amount of CO_2 fixed (or taken up) by the process of photosynthesis less the amount of CO_2 evolved by the processes of dark (or mitochondrial) respiration and photorespiration (a process whereby some of the products of photosynthesis are re-oxidised by a different metabolic pathway to that of mitochondrial respiration). Net CO_2 exchange is therefore more easily measured than are these three components of CO_2 exchange.

This report describes the design and construction of a system, incorporating CO_2 and H_2O IRGAs, at WRO.

Previous designs of IRGA systems and people consulted

IRGA systems have been widely used in contexts similar to those discussed in this report.

Information used in the early stages of design was obtained largely from reports by (1) Van Oorschott and colleagues at Wageningen, (2) various workers at G.R.I. Hurley, (3) Parkinson and colleagues at Rothamsted, (4) an I.I.T.A. group at Reading University. A bibliography of reports consulted appears in Appendix 2.

In addition to this literature, visits were made (G.R.I. to George Ryle), Rothamsted (Dr Keith Parkinson and Dr David Lawlor), and also to A.D.C. Ltd who manufacture IRGA equipment (Mr Ernest Moult).

The following points emerged from our survey of the literature and discussions:

The essential components of an IRGA system are: 1. A supply of air of known CO_2 and H_2O content and at a known flow rate; 2. One or more cuvettes -

containers which house the biological material; 3. The Analyser; 4. A system for recording the data. In addition if more than one treatment or replicate is required (therefore more than one cuvette) we can add - 5. A system for selectively sampling the cuvettes.

There are 3 main types of IRGA system according to air supply: 1. CLOSED system, in which no air enters or leaves the system, except for a sample which flows to the IRGA, is measured, and returned to the cuvette. 2. SEMI-CLOSED system, in which changes in CO_2 concentration in a closed system are compensated by controlled addition of CO_2, CO_2-enriched air or CO_2 depleted air. 3. OPEN system in which air is either drawn from outdoors or supplied by cylinders, is passed through the cuvette then passed out to exhaust.

Most systems in use are of the open type. Small biological material can be dealt with by a cylinder supply, but larger material requires too high a throughput and necessitates the use of outside air. This can either be measured for CO_2 (and H_2O) content, or modified using CO_2 absorbers and a supply of pure CO_2 (with appropriate control gear). Water content is controlled by normal humidity control systems, such as cooling to a dewpoint followed by re-heating. An important factor is that most IRGAs can be used either in an absolute mode (measuring CO_2 in ul/l from zero to a given value) or in a differential mode, where the sample of unknown air is compared to a sample of the supply air, and the difference in CO_2 concentration determined. The differential mode is normally used where there may be slow cyclic changes in supply air CO_2 concentration, as is normally the case with outdoor air. In such a system, short term fluctuations in supply air CO_2 content are overcome by the use of mixing chambers in the supply line.

The component of the system which requires most consideration in design is the cuvette itself. Due to the wide range of biological material used (from small leaf sections to areas of turf in the field) cuvettes must be designed in size and shape to fit the task. This part of the system is normally designed entirely by the individual researcher and flexibility is difficult to achieve. Cuvettes described in the literature and observed on our visits, varied from simple open-ended glass tubes to large perspex structures for small field plots. The system described by Louwerse & Van Oorschott (1969) has a complexity which exceeds that of a Saxcil growth cabinet.

Objectives of our design

The ability to measure CO_2 and H_2O exchange continuously provides useful non-destructive data on dynamic aspects of herbicide action in relation to environmental changes. Most previous work has relied on some destructive end point assessment such as plant weight, or a phytochemical determination.

The system was designed to be as flexible as possible, in order to cope with plant material ranging from small seedlings up to large, tillered grasses such as Elymus repens. The cuvette design had to allow for the separation of foliage from soil and roots, since these would interfere with the gas-exchange measurements. For larger plants, the required airflow would be too high for a cylinder supply to be feasible, so a system using outdoor air was required. The temperature and humidity of outdoor air are variable and are adjusted by passing air from outside into a Saxcil controlled environment cabinet. It was decided to opt for a negative pressure system, drawing the airflow through the cuvettes by a suction pump. This has the disadvantage over a positive pressure system that any leaks into the system may admit air with very high

concentration of CO_2 if people are working around the apparatus. This does not happen with positive pressure, but it was found that pumping caused an unacceptible rise in temperature of the air which would necessitate further complications in control of temperature and humidity. It was felt that suitably reliable leak-proof seals could be made.

In order to overcome variations in CO_2 and water vapour exchange due to differences in plant material, many workers have carefully controlled the area of the leaf sampled and its orientation with respect to light, and in some cases leaf discs have been used. However, in our design it was decided to accept a degree of variation as being inherent in plant material and to allow for this by replication. Thus the system was designed to permit up to 12 replicate plants or units of plant material, although usually only 10 cuvettes are available due to the calibration system employed.

The system allows intermittent recording (1-59 min., intervals) for up to 12 samples, with data collection and processing by a microcomputer.

DESCRIPTION

General layout (see figure 1).

The system comprises the following unit:

a) The sample cuvettes.
b) An airflow system, drawing air through the cuvettes.
c) A gas sampling unit, drawing a sample of air from the cuvettes for analysis.
d) CO_2 and H_2O Analysers (IRGAs).
e) The microcomputer control system (not shown in figure 1).

The system was initially designed for use with sample cuvettes housed in a Saxcil cabinet, although it was envisaged that work in other locations would be possible (e.g. a glasshouse), given the availability of a means of controlling the temperature and humidity of air drawn from outside, at a reasonably stable CO_2 concentration.

Sample cuvettes

The cuvette design used in experiments with young chickweed plants (Stellaria media) is shown in Figure 2. This consists of a clear polystyrene box (150 x 100 x 75 mm) with a push-fit lid. The box is used in an inverted position, with the lid adapted to allow a polystyrene cup (75 mm diameter at open end x 82 mm high) to be clamped to it. The soil or compost is held in the cup, and pre-germinated seed are sown in this soil so that the developing shoot will emerge through a plastic grommet which was fixed to the lid. The grommet was made by driving out the metal insert from a 4 mm polypropylene panel-mounting insulated socket (R.S. Components 444-618). The emerged shoot can be sealed in the polystyrene box using non-phytotoxic rubber preventing ingress of CO_2 and H_2O particularly from the soil and roots. Two outlets are provided for the main airflow and sample lines, and air enters the box through a hole in the opposite wall. Small leaks into the box were considered unimportant, since the box was surrounded by the ambient air, and tests showed that most of the air entered via the inlet hole. However, if necessary the box could be sealed by PVC tape around the junction of the base and lid. Air within the cuvette is constantly stirred by a small 6V D.C. electric fan (Micronel V241L).

Although this design of cuvette was used for the first experiments, other designs have been used and the system was designed to suit a range of airflows, suitable for a range of cuvette sizes.

The Airflow System and Sampling Unit. (Refer to figure 1).

The sample cuvettes are placed in a Saxcil cabinet set at the required temperature and humidity. Air supply to the cabinet is drawn from a roof top air intake outside the building, via a mixing tank of 230 l volume to avoid short-term fluctuations in CO_2 concentration.

The airflow through the cuvette is drawn by pumps (Charles Austin, Duplex 44) via separate flowmeters (Meterate R53) and needle valves (Nupro SS-4MG). A sample of the air from the cuvette is drawn continuously through a separate sample line to a sampling unit (ADC WA 161/12/A). This unit selects between 12 separate flow lines and passes the selected line out for analysis, whilst the remaining 11 lines are kept flowing at the same rate via a by-pass system. Each line has its own 3-way solenoid, flowmeter and needle valve, with extra needle valves and flowmeters for the sample line to the anlyser and a reference line. Switching for selection of the sampled solenoid can either be operated manually or automatically by selecting the number of channels and the dwell time. Alternatively the unit can be externally controlled by a microcomputer fitted with an interface to read the channel number and operate the channel selector.

The IRGAs

The sample and reference lines are pumped by the sampling unit, and are piped to two infra-red gas analysers. The first is an ADC Mk II fitted for water vapour measurement, and the second an ADC Mk III fitted for CO_2 measurement. In both analysers the analysis cell is divided into a small cell (5% of the total length) and a large cell (95%). The sample line passes through the small analysis cell of the water vapour analyser, which is used in absolute mode, and then on to the large analysis cell of the CO_2 analyser, which is used differentially. As an additional refinement to the analysis system two 3-way solenoids are fitted which make it possible to automatically calibrate the CO_2 analyser at any selected interval. Thus zero and sensitivity can be checked as they vary with changes in the CO_2 concentration of the ambient air. The two valves are placed in the sample line, between the large and small analysis cells in the CO_2 analyser. Other lines enter the solenoids from the CO_2-free air (pure air) outlet of the analyser and from a cylinder of air with known CO_2 concentration around 300 ppm. The microcomputer controls the operation of these valves so that an autocalibration sequence may be performed at any time in the sampling sequence, and the analyser zero and sensitivity, as well as the ambient air CO_2 concentration, can be determined according to the equations of Parkinson and Legg (1978).

Calibration of the water vapour IRGA is achieved using a water vapour generator (ADC, WG 600) at the beginning of each experiment.

Sampling control and data handling

The gas sampling unit has an internal channel stepping system, allowing successive channels to be sampled, and the output voltage from the analyser to be recorded (e.g. on a chart recorder) as a series of pulses whose amplitudes are proportional to the analyser output from these channels. This is sufficient for some purposes, but collecting the information from the

recording is tedious and error prone, and the options available for varying sample sequence and dwell time are very limited. We therefore decided to use a microcomputer to control the sampling, and to scale and store the data.

The system uses an Apple 2 computer, because these machines are used extensively in WRO, and therefore subsequent processing of the stored information could be done elsewhere, without occupying the analyser's own machine. There are no features which could not be implemented on a similar computer of another make, although of course the detail of the programs and the peripheral hardware would differ.

Software outline

The main features of the control program are:

1. The user has flexible control of the order of sampling, the time interval between samples, the measurement mode of the analysers and whether either or both of the two analysers are to be interrogated. The control parameters may be stored in a disc file for subsequent re-use.

2. Calibration span (sensitivity) and zero data for each analyser can be automatically calculated by sampling zero and standard gases. This calibration is then applied to each measurement before display and storage. Automatic calibration is possible each sample cycle for the CO_2 analyser. Non-linearity in the H_2O analyser is corrected before data storage or display.

3. Single channels may be sampled rapidly and the readings displayed for purposes of setting up the system. Successive samples of a single channel can be displayed as a graph to observe stability or equilibration rate. There is an option to store the readings on disc or to print the graph on the printer.

4. In normal sampling mode, each measurement is stored in a disc file together with the channel number and elapsed time. Files may be catalogued and deleted from within the program, and a new data file may be started at any time. Files may be inspected by printing the contents. An offline file handling program contains further file manipulation facilities and can create new files containing the information from a single channel. A choice of formats is available to suit the different requirements of other programs, including a format which can be plotted on a high quality pen plotter.

System components

The computer system comprises these devices:

Apple 2+ computer, 48k memory.
Two 5.25" disc drives.
Epson MX80 printer.
U-microcomputers U-TIM elapsed time clock.
U-microcomputers U-BCD input/output card.
MC Computers A to D converter.

The BCD card is used to read the channel number of the ADC WA 161 gas sampling unit, and also outputs stepping pulses to the unit to change channel. The correct number of pulses is generated by software which compares the current channel with the required one.

The timer card functions like a stopwatch, having 'go', 'stop' and 'reset' commands implemented by writing 1's into the appropriate bits of a control

memory location. Other control bits are used to cause interrupts at specified time intervals. The timer has a maximum time of 59 minutes 59 seconds, but a 1 hour interrupt can be used to drive a software hours counter.

The A to D converter is a 12 bit 16 channel device, of which the first two channels are dedicated to the two analyser outputs. The other channels are available for further expansion, for example temperature measurements in the plant chambers.

The printer has an 'intelligent interface' card which can print a hard copy of the graphics screens with a single command. It has an extended instruction set including a Fortran-like format routine for presenting numbers in fixed format.

Particular software features

It is not practical to describe the entire program in detail, but it is of value to look at some specific features.

The program is written in Structured Basic, an enhanced Basic which supports named subroutines, local variables, repeat..... until loops and other features which make the program easier to write and modify. It does not use line numbers, so library subroutines can be used easily. It is a good compromise for a computer which does not have a structured language as standard, offering many of the advantages with no additional hardware or loss of facilities.

The program is a menu-selected set of subroutines, plus a very small routine called STARTUP which is executed once at the beginning of the program to set initial conditions, array bounds and so on. STARTUP also executes a default parameter setting routine. If a user does not wish to set his own parameters, the program will provide a default package which scans each channel, both analysers, with a sample interval of one minute. Calibrations are set to match the analysers' meter indications and a default file name of OUTPUT is declared. This default package allows the inexperienced user to start recording immediately without complications which might arise if parameters were not set to usable values.

Each sample measurement is stored in a random access file record, with its channel number and time. The original version used sequential files, but this proved unworkable, as the computer has to read the entire file before appending the new record, a process which can take longer than the sample interval for long files.

Screen displays

The Apple 2+ has only a small screen memory (40 characters - 24 lines) so the amount of information that can be displayed at one time is limited. The standard display when the machine is in normal scanning mode shows the elapsed time, current file name, autocalibration on/off, current and last channels. Above this information is shown the latest and two previous measurements of each channel in the scan sequence.

When the program is in manual single channel mode a rapidly updated measurement of the chosen channel is displayed, together with the channel number. The channel being sampled may be changed at will by use of the < and > cursor keys.

The fast single channel mode makes measurements at 3, 10 or 60 second intervals and draws a graph of the latest 50 samples on the screen. Each new measurement 'pushes' all the previous ones back, so the effect is of looking through a window onto the moving chart of a chart recorder. The user can press 'P' to print a copy of the screen display, or can select an option to have this done automatically every 50 measurements.

Automatic calibration

Automatic calibration of the CO_2 analyser in differential mode is done by the method of Parkinson and Legg (1978). The method depends on passing a zero (CO_2-free) gas and then a standard gas through the 5% cell of the analyser (see Appendix 3). To do this under computer control, two extra solenoid valves, SV1 and SV2 were fitted to the WA161 sampling unit. SV1 allows an external source of gas to be inserted in the 5% cell in place of the normal sample, and SV2 selects whether this will be the zero or the standard gas.

Autocalibration is switched on or off by options in the calibration menu. When autocal is on, a flag is set, and whenever channel 12 is scanned, this flag is inspected. If it is set, the autocalibration sequence is started. Valves SV1 and SV2 are controlled by two of the computer's 'annunciator' outputs via optoisolators. In autocal mode, channels 1 to 11 are stored as differences from channel 12, which is normally allocated to the background or reference air. The absolute value of channel 12's CO_2 concentration is stored on the disc to give an indication of the ambient level. The A-to-D readings from the zero and standard gases are also stored as diagnostic information in case of errors or faults.

The ability to perform a calibration once every sample cycle ensures that errors due to zero drift or changes in sensitivity (even those arising from inadvertent changing of the analyser's gain and zero controls) are automatically corrected within one cycle, provided that the measurements remain within the reading range of the A to D converter.

Automatic restart after power interruption

The system can store files of operating parameters such as sample sequence, sample inteval, calibration information and output file names in a parameter file on the program disc. Any number of parameter files may exist at once, up to the maximum capacity of the disc, each file being identified by a user-given serial number. Any file with serial number 0 has a special significance, as it is used by the program's auto restart facility which allows operation to resume after a power cut. On restoration of power, the main program is loaded from disc, goes through an initialising routine, then attempts to read parameter file 0. If it succeeds, it starts scanning using the parameters it has just read. If it fails, i.e. file 0 is not present, it presents the main menu and waits for user instructions.

Offline data handling

A separate suite of programs, though with a common base of subroutines and variable names, is provided for handling the information collected by the main program. It can be run on any Apple computer equipped with disc drives and a printer.

It can catalogue and delete files, print portions of files, or create subfiles from the original file. Files to be processed can be queued for attention by the program, thus freeing the user from returning to the keyboard

until the stack of files has been dealt with. For each input file in the queue, the user specifies which channels and which gases are to be put into subfiles. As each subfile is completed, a message is typed on the printer giving the source and destination file name and the number of items transferred to the new file. Destination file names are allocated automatically by the computer by linking the source file name with the gas and channel identification. Files intended for plotting by a pen plotter have the file name modified to suit the particular requirements of that program, and also carry a header record indicating the number of items in the file.

OPERATION

Preliminary tests and calibration.

a) Ambient air variation.

Initial measurements during testing of the equipment revealed that the CO_2 concentration of the air in the Saxcil cabinet closely followed that of air taken directly from outside the building. The concentration showed a marked cycling between night and day, with a range of by up to 50 ppm. This emphasised the need for regular calibration.

b) Response time.

Tests were performed to show the time taken for a change in rate of CO_2 exchange in the cuvettes to be detected by the analysers. To do this, a chickweed plant was allowed to equilibrate in a cuvette. When the test started readings were taken of CO_2 exchange every minute. After 10 minutes the cabinet lights were switched off, and after a further 10 minutes switched on again. The resulting data (Fig. 3) showed that within 3-5 minutes the new rate of CO_2 exchange had reached equilbrium after switching off the lights. Upon switching on the lights again the change was less rapid. The rate of change in CO_2 exchange detected is a consequence of both the physiologicl changes associated with the plant and the time taken to achieve mixing of the air in the cuvette and transit to the analyser. Since the combined effect results in less than 5 minutes for complete changeover when switching off the lights, the delay in detection of a real change in CO_2 exchange must be somewhat less than this, and therefore such a delay is unimportant in relation to typical sampling intervals in experiments of 30 minutes or more. The slower response to switching on the lights after a period of darkness is thought to indicate stomatal aperture changes.

c) Boundary layer resistance.

Another feature of the apparatus which required evaluation was the effects of airflow and mechanical stirring on the boundary layer resistance to gaseous diffusion around the leaf surfaces. The boundary layer is disturbed by turbulence in the cuvette, and since some turbulence is inevitable due to air flow it is necessary to make sure that boundary layer resistance (i.e. the resistance to vapour transfer which is due to the boundary layer) is reduced to a level at which its effect on the rate of CO_2 exchange is negligible in comparison with the turbulent mixing effect. If this is achieved the individual aerodynamic differences between cuvettes and plants can be ignored, and furthermore the rate of airflow through the cuvette can be varied according to plant size, thus giving a desired depletion of CO_2, but without affecting the efficiency of gaseous transfer. A value of 0.1 sec cm^{-1} is given by Sestak, Catsky and Jarvis (1971) as the boundary layer resistance typical of a well-stirred chamber.

The boundary layer resistance in the cuvettes shown in Figure 2 was determined in the manner described by Woodward & Sheehy (1983). Evaporation of water from a wet absorbent disc (such as blotting paper) is measured over a period of time. From a knowledge of the flux of vapour transfer and the difference between the ambient vapour concentration and the saturated vapour pressure at the evaporating surface, the boundary layer resistance can be calculated by a simple Ohm's Law analogy:

$$r_b = \frac{Ca - Cs}{F}$$

where r_b = the boundary layer resistance (s m^{-1})
Ca = the absolute humidity in the ambient air (g H_2O m^{-3})
Cs = the absolute humidity of the saturated vapour at the evaporating surface (g H_2O m^{-3})
F = Flux of water vapour transfer per unit area of evaporating surface (g H_2O m-2 S^{-1})

Note that to calculate Cs the temperature of the evaporating surface must be measured. This is done by having a fine thermocouple in contact with the evaporating surface. Using this the depression of temperature caused by evaporation can be measured.

This method was used to measure the boundary layer resistance of evaporation from 20 mm diameter glassfibre filter pads (Millipore AP 2502000). These were mounted on fine glass capillary tubes so that they could be kept saturated with water from a small reservoir. Four such discs were mounted on a single reservoir, the reservoirs being made from the end 25 mm or so of 5 ml disposable plastic syringes. The evaporation of water from the whole assembly was measured for 15 minute periods, and the loss of water from the reservoir calculated from the weight before and after the experimental period.

Results of the measurement of boundary layer resistance are shown in Table 1. The values obtained are considered adequately small for the purposes of this system as outlined above.

Table 1. Calculated boundary layer resistance (r) to water vapour transfer in small plant cuvettes (as shown in Fig. 2)

Ambient temperature (ingoing) °C	Ambient humidity (ingoing) °C	Air flow rate 1 min^{-1}	Fan circulation off/on	Boundary layer resistance (r) scm $^{-1}$
15.6	76	1.5	ON	0.69
13.7	87	1.5	ON	0.88
14.2	84	0.5	ON	0.83
14.2	84	0.5	ON	0.97
13.9	86	1.5	OFF	1.72

NOTES:

1) Absolute humidity in the cuvettes, used for these calculations, a mean of those of the ingoing and outgoing air.

2) Note the high value of r in the absence of stirring (fans off) and the relative independence of r and flow rate with the fans on.

Experimental procedure

This section is intended as a step-by-step guide for using the IRGA system, though it should be noted that certain details may be changed as improvements are made.

This guide assumes that the system is not dismantled and that the operator has set up the Saxcil cabinet conditions and prepared plant material suitably for a set of cuvettes.

Preparation

1. Change all absorbent column materials if not recently done. The external silica gel columns will need changing at least every two days when in use.

2. Check the gas pressure in the dry air and standard CO_2 cylinders. Change the cylinders when they have less than 30 bars pressure.

3. Check all piping and electrical connections.

Analysis

4. Switch on the IRGAs to warm up. This must be done at least 2 hours before use, but preferably the day before. Check all pipe connections to the irga cells (see diagrams in manuals if in doubt).

5. Switch on the by-pass pump, situated in the base of the sampling unit/IRGA stack. Also switch on the sampling unit and the main airflow pump (or pumps if more than one needed).

6. Microcomputer. Insert the program disc in drive 1 and switch on the microcomputer (make sure there is an empty or unfilled initialised disc in drive 2 for data storage).

   If there is a Params 0 file on the Analyser Programme disc, this will load and sampling will commence. Stop this with [Control-P]. The VDU Will now display the user menu.

   Select the calibration option first, and set up the IRGAs and computer instructions for calibration (see 7 below). Then select the option "select/review parameters" and set up the parameters required for the experiment. Note that if automatic start is required in the event of power failure the parameters must be stored as Params 0 (with a suitable filename, such as "Restart" so that the original data file is not overwritten). If more than one power failure occurs the most recent file with the re-start name will be saved at the expense of previous re-start files. When all parameters are set, and the desired output filename chosen, return to the menu. At this point you are ready to start sampling so the cuvettes and plant material must be prepared and any treatments carried out. It is advisable to reset the clock at the last moment before starting the sample programme. Finally select the "start/resume sampling" option.

7. Calibration

a) CO_2. Zero and gain controls on the IRGA should be checked and if necessary coarsely set to give zero (differentially) at about + 10 ppm on the differential scale (reference air passing through both analysis and reference cells). Sensitivity can be checked by passing CO_2-free air through the 5% analysis cell. However, precise calibration of the CO_2 analyser is unnecesary since the autocalibration routine will do this during the experiment. In the computer "set calibration" routine simply select "Autocal on" and key in the appropriate standard gas concentration (which is otherwise set to a default value around 300 ppm).

b) H_2O. Switch on and set the water vapour generator at least 2 hours before calibration. The temperature should be set to 28.9°C which will produce 25 m bar water vapour pressure (i.e. full scale deflection of the IRGA), with the water vapour generator outlets set to 100% emission. Turn on the dry air cylinder 15 minutes before calibration, and set the pressure gauge to 1.4 bars, using the regulator valve on the cylinder. Calibration is achieved by passing the standard water vapour through the small analysis cell and setting the sensitivity with the gain control, whilst alternatively passing dry air through the cell and setting the zero. With the computer "set calibration" option selected, select the option "set calibration by zero and measurement". This option is self explanatory.

Example results

As an example of the type of experiment which has been conducted using this system, some results are presented from an experiment with *Stellaria media* plants treated with ioxynil.

Method

S. media plants were grown in nutrient solution held in polystyrene boxes (58 x 38 x 22 mm) such that their foliage was outside the box and the roots and nutrient solution sealed inside. Six replicate plants were sprayed with 0.4 kg/ha ioxynil ester and two plants were left untreated. The plants were then placed in the IRGA system cuvettes and carbon dioxide and water vapour exchange monitored over a period of 5 days.

Results

Fig. 4 shows the CO_2 exchange with respect to time after spraying. The two lines are the mean values for the treated (TR) and unsprayed control (CON) plants respectively. The pronounced 'square-wave' pattern of oscillation in the control plants represents the alternation between net respiratory CO_2 evolution during the night period and net CO_2 fixation during the day period. The treated plants can be seen to lose most of their photosynthetic ability during the first 24-48 hours. Shorter term fluctuations in readings are partly due to systematic errors in the equipment and instruments, and partly due to biological variations, such as variations in stomatal conductivity due to fluctuations in air humidity.

The between-replicate variability of the treated plants can be seen in Fig. 5.

Water vapour measurements (Fig. 6) are recorded in absolute rather than differential units and must therefore be compared with the ambient vapour pressure (AMB). FIG. shows how this ambient vapour pressure fluctuates over a relatively short time and also varies between day and night periods (indicated by vertical lines on the graph). Transpirational water loss is represented by the difference between the lines for the treated or control plants and the ambient vapour pressure. As Fig. 6 shows, there was little effect of ioxynil on water vapour exchange in this experiment despite substantial changes in CO_2 exchange induced by the herbicide.

Graphs such as those shown in Figs. 4-6 are rapidly produced from the stored data. Further analysis is possible by transferring this data to a mainframe computer, or by printing data tables and doing smaller manual computations on selected data.

APPENDIX 1 - List of suppliers

Supplier	Product
Air Products Ltd Special Gases Dept. Doncastle Road Bracknell Berks RG12 4LH	Standard gas mixtures; CO_2 (specified ppm) in 20% oxygen in nitrogen.
The Analytical Development Co Ltd Pindar Road Hoddesdon Herts EN11 OAQ 09924 69638	IRGAs, gas sampling unit, water vapourgenerator
BDH Chemicals Ltd Fourways Carlyon Industrial Estate Atherstone Warwickshire CV9 1JQ 082 77 3631	Ferrous sulphate magnesium percholorate silica gel (self-indicating) soda lime (self-indicating)
Charles Austen Pumps Ltd 100 Royston Road Byfleet Weybridge Surrey KT14 7PB Byfleet 43224	Air Pumps
Comark Ltd Rustington Littlehampton Sussex BN16 3QZ	Hand held thermometer and miniature thermocouples
Glass Precision Engineering Ltd Mark Road Hemel Hempstead Hertfordshire (0442 56371)	Meterate flowmeters
Isis Pneumatics Ltd 477, Malton Avenue Slough, Berks	Norma plastic tube connectors
North London Valve & Fitting Co Ltd 34 Capitol Way Capital Industrial Park London NW9 OEQ 01-200-1677	Whitney and Nupro valves

Payne Scientific
6 Iveley Road
London SW4 OHS
01-720-5801

'Portex" vinyl tubing
'Unex" hose clips

Radiatron Ltd
Crown Road
Twickenham TW1 3ET
01-891-1221

Micronel miniature fans

R.S. Components Ltd
PO Box 427
13-17 Epworth Street
London EC2P 2HA

Electronics components
Sundry nuts, bolts etc.
Power supply for autocalibration
solenoid valves

Stewart Plastics Ltd
Purley Way
Croydon

Polystyrene boxes and
containers

Computing and software

Oxford Data Systems
29 Pound Way
Cowley Oxford
(or any other Apple supplier)

Apple II computer and disc drives
Epson MX80 printer and interface card

U-Microcomputers Ltd
Winstanley Industrial Estate
Long Lane Warrington
Cheshire WA2 8PR

U-BCD input/output card
U-TIM Interval timer
'Structured Basic' language
(U-microcomputers can also supply a rack mounted computer similar to an Apple 2e)

Verospeed Ltd
Standard road
Boycott Wood
Eastleigh
Hants SO5 4ZY

Optoisolators and solenoid drivers
for auto calibration valves

MC Computers Ltd
West Mills
Newbury Berks

A to D converter card

APPENDIX 2 - BIBLIOGRAPHY AND REFERENCES

Hadley, P., Boxall, M.I., Richardson, A.C., Dickinson, D., Mincham, F.R., Summerfield, R.J., and Roberts, E.H. (1979) A system for continuous monitoring of whole shoot CO_2 exchange as an adjunct to growth analysis experiments. Report of the Reading University International Institute of Tropical Agriculture, Tropical Grain Legume Physiology Project.

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Parkinson, K.J. (1968) Apparatus for the simultaneous measurement of water vapour and carbon dioxide exchange of single leaves. Journal of Experimental Botany, 19, 840-856.

Parkinson, K.J., Day, W. and Leach, J.E. (1980) A portable system for measuring the photosynthesis and transpiration of graminaceous leaves. Journal of Experimental Botany, 31, 1441-1453.

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Sestak, Z., Catsky, J. and Jarvis, P.G. (1971) Plant Photosynthetic production. Manual of Methods. Dr W. Jink NV (Publ.) The Hague.

Stiles, W. (1977) Enclosure method for measuring photosynthesis, respiration and transpiration of crops in the field. Technical Report No.77, G.R.I., Hurley.

Woodward, F.I. and Sheehy, J.E. (1983) Principles and measurements in environmental Biology. Butterworths.

APPENDIX 3 - THE CALIBRATION OF CO_2 ANALYSERS BY THE METHOD OF PARKINSON AND LEGG (1978)

This method of calibration requires four separate readings taken from the analyser with gas flows set up as follows:

Analyser reading	Long (95%) Analysis tube	Short (5%) Analysis tube
R.1 (measurement)	Unknown	Unknown
R.2 (zero)	Reference	Reference
R.3 (calibration)	Reference	CO_2 free air
R.4 (calibration)	Reference	Standard CO_2 mix

With reference air passed through the reference tube in each case.

These four readings permit the calculation of the analyser sensitivity (S), the absolute concentration of the reference air (C_R) and the concentration of the unknown sample undergoing analysis (C_A) as follows:-

$$C_R = C_S \times (R2 - R3)/(R4 - R3)$$

$$S = (R4 - R3)/(CS \times 1_2/(L_1 + L_2)$$

$$C_A = C_R + (R1 - R2)/S$$

where C_S = Concentration of standard CO_2 mix

L_1, L_2 = lengths of long and short analysis tubes respectively.

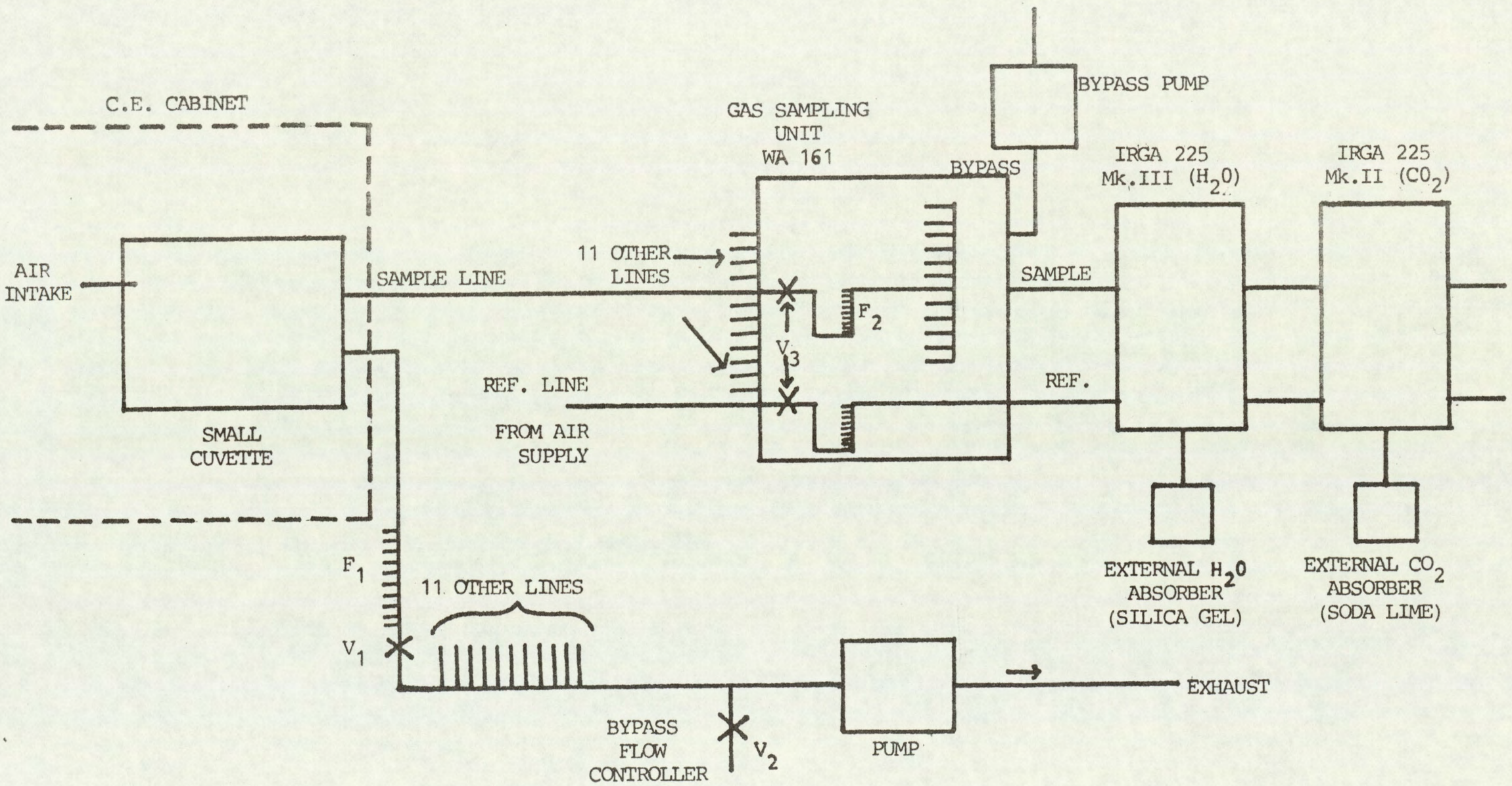


Fig.1. Gas Flow System for up to 12 small cuvettes housed in a C.E. cabinet.

F_1, F_2 = flowmeters; V_1 - V_3 = flow control valves.

IRGA System : Plant Chamber.

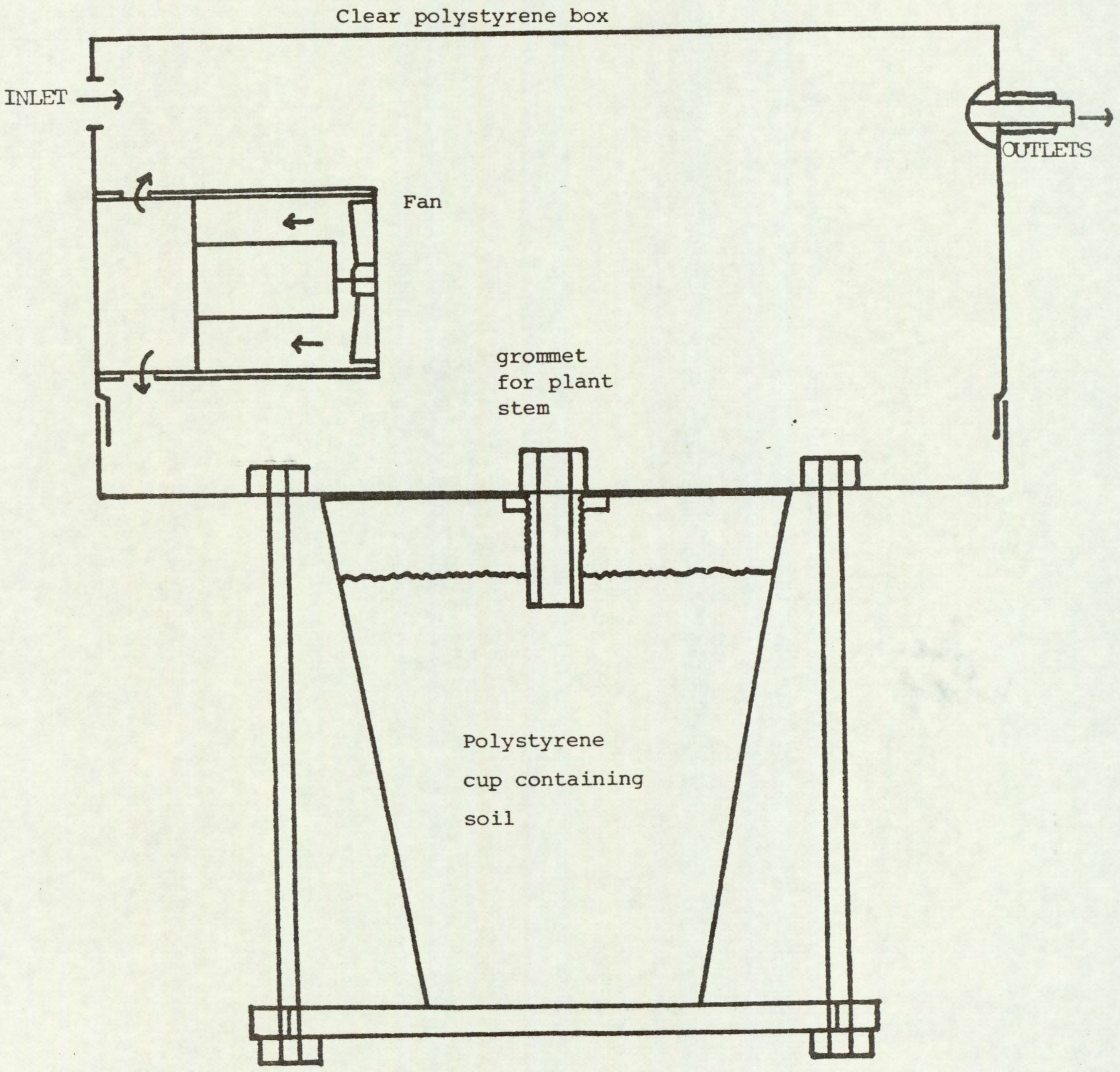


Fig. 2. Small sample cuvette
(approximately actual size)

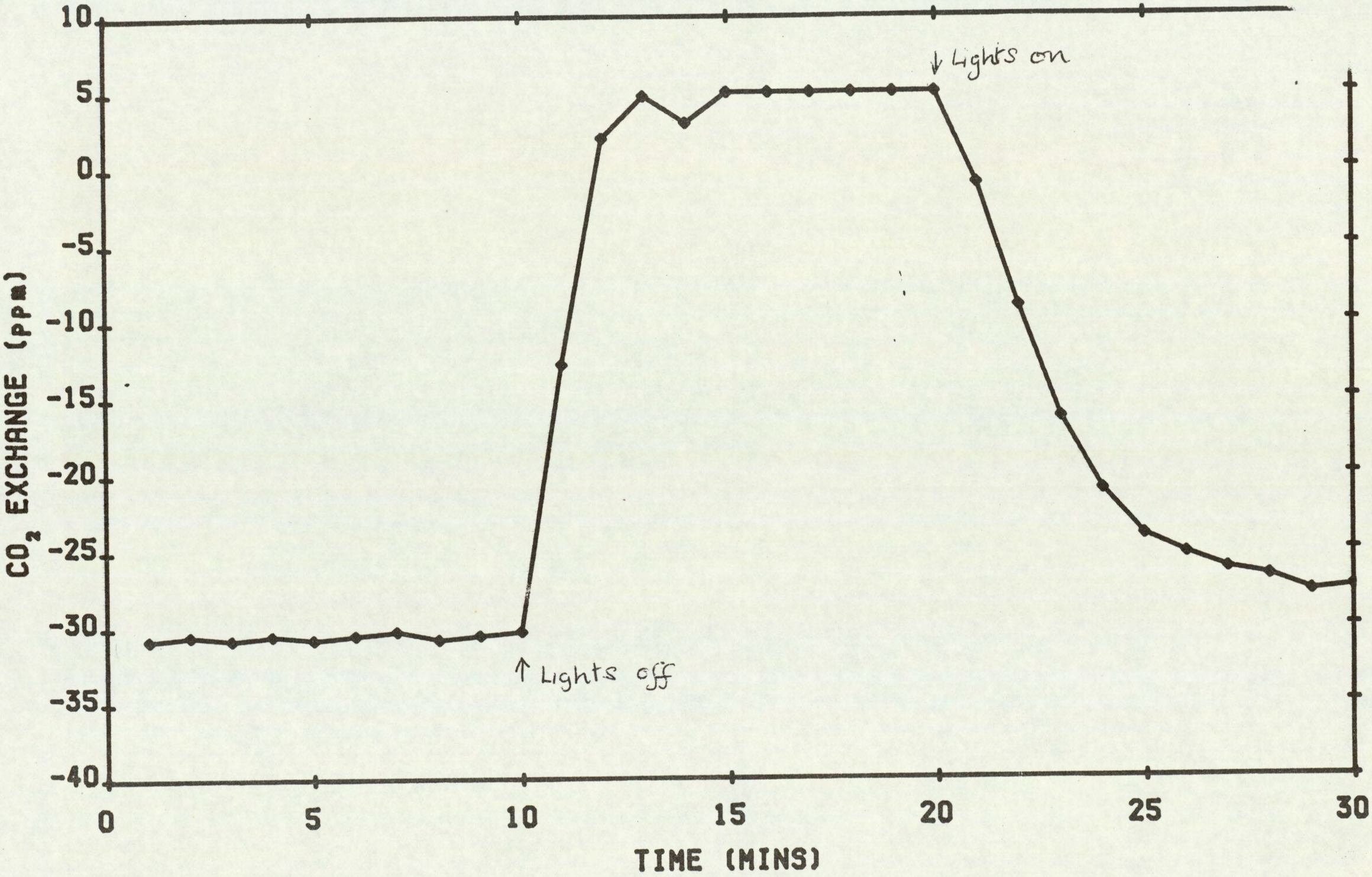


Fig. 3. IRGA system response time.

Fig. 4. Carbon dioxide exchange for means of six replicate plants S. media grown in nutrient solutions. CON = unsprayed control; TR = Treated by spraying with 0.4 kg/ha ioxynil octanoate.

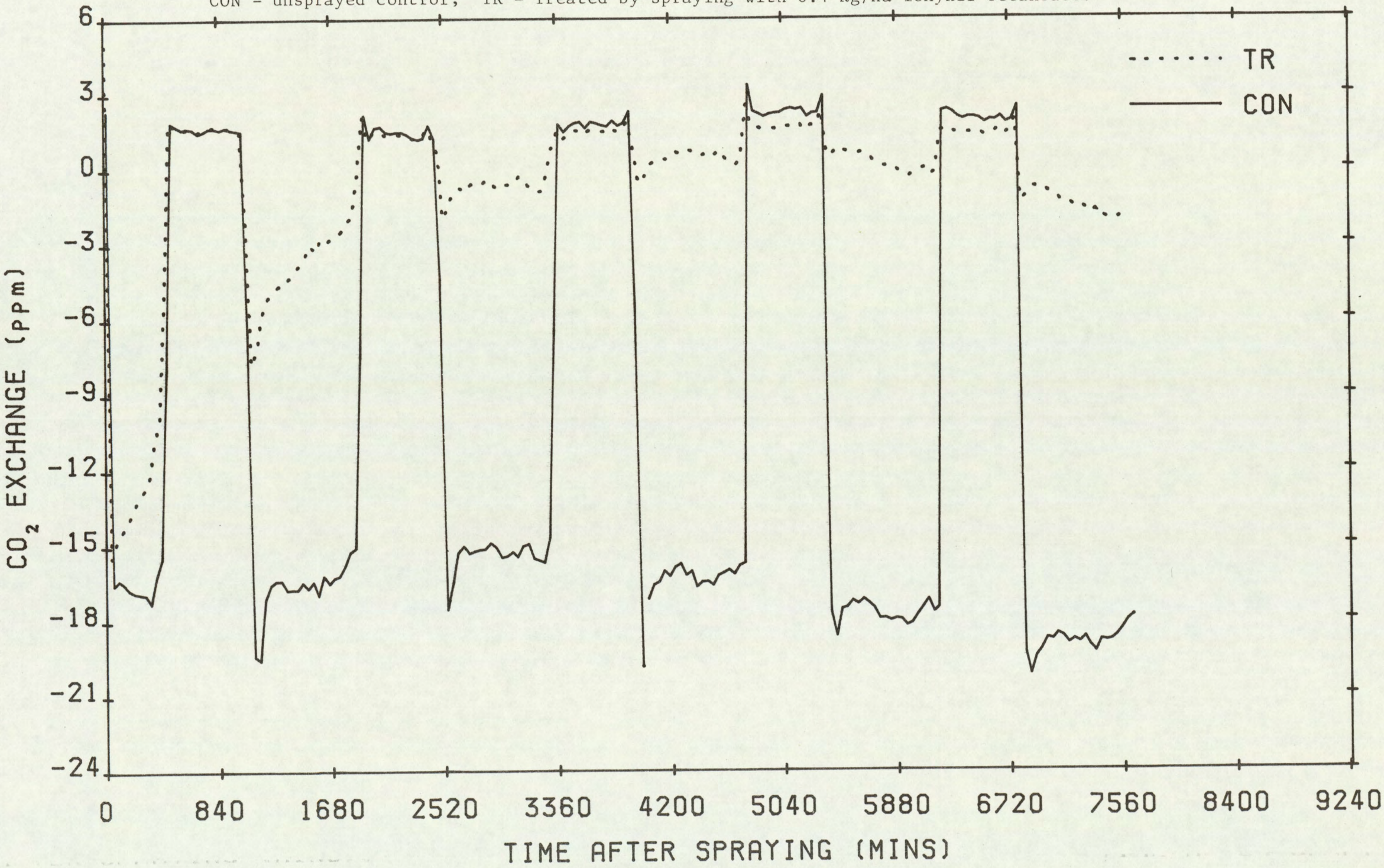


Fig. 5. Carbon dioxide exchange for six individual plants (S. media) after spraying with 0.4 kg/ha ioxynil octanoate.

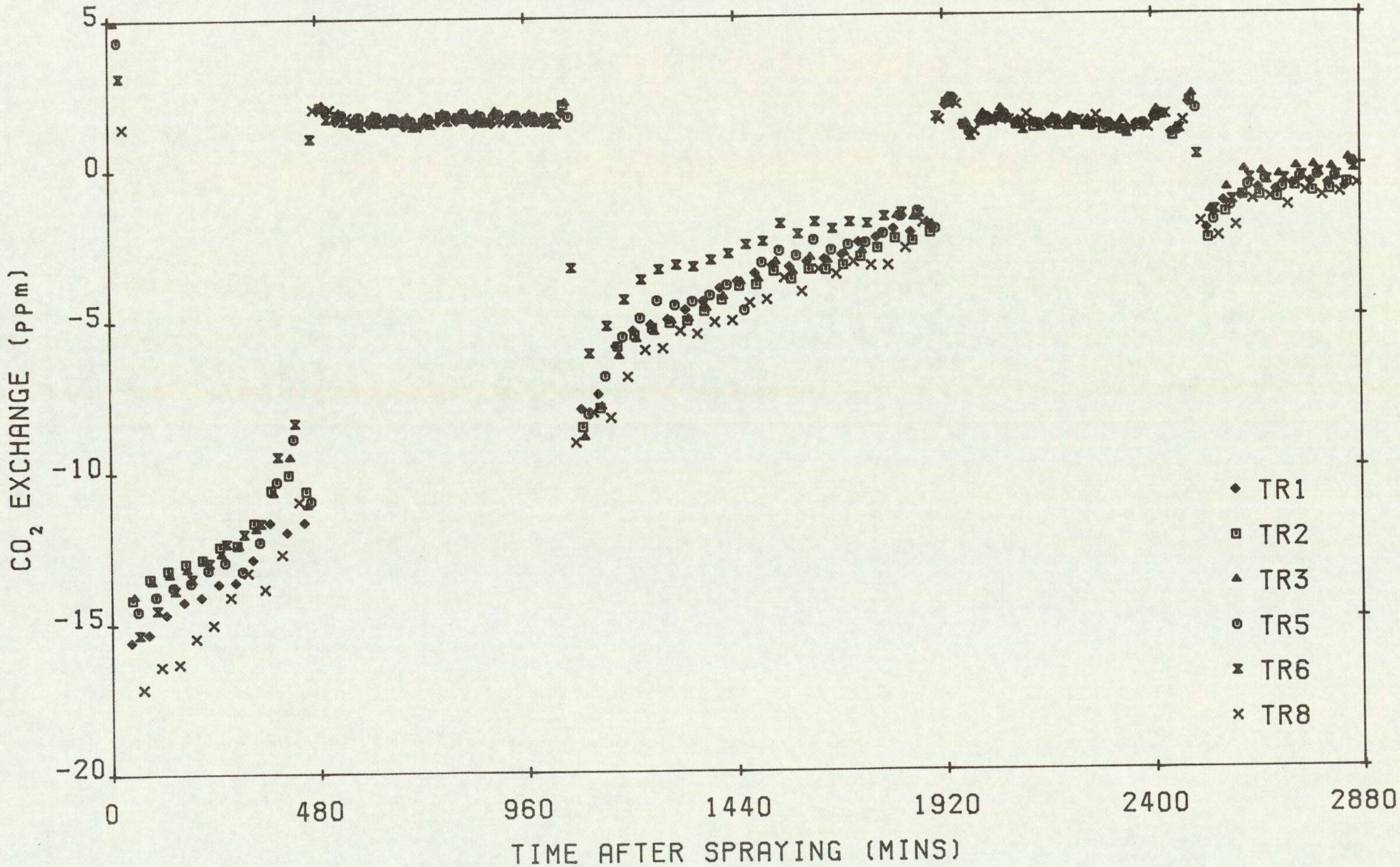
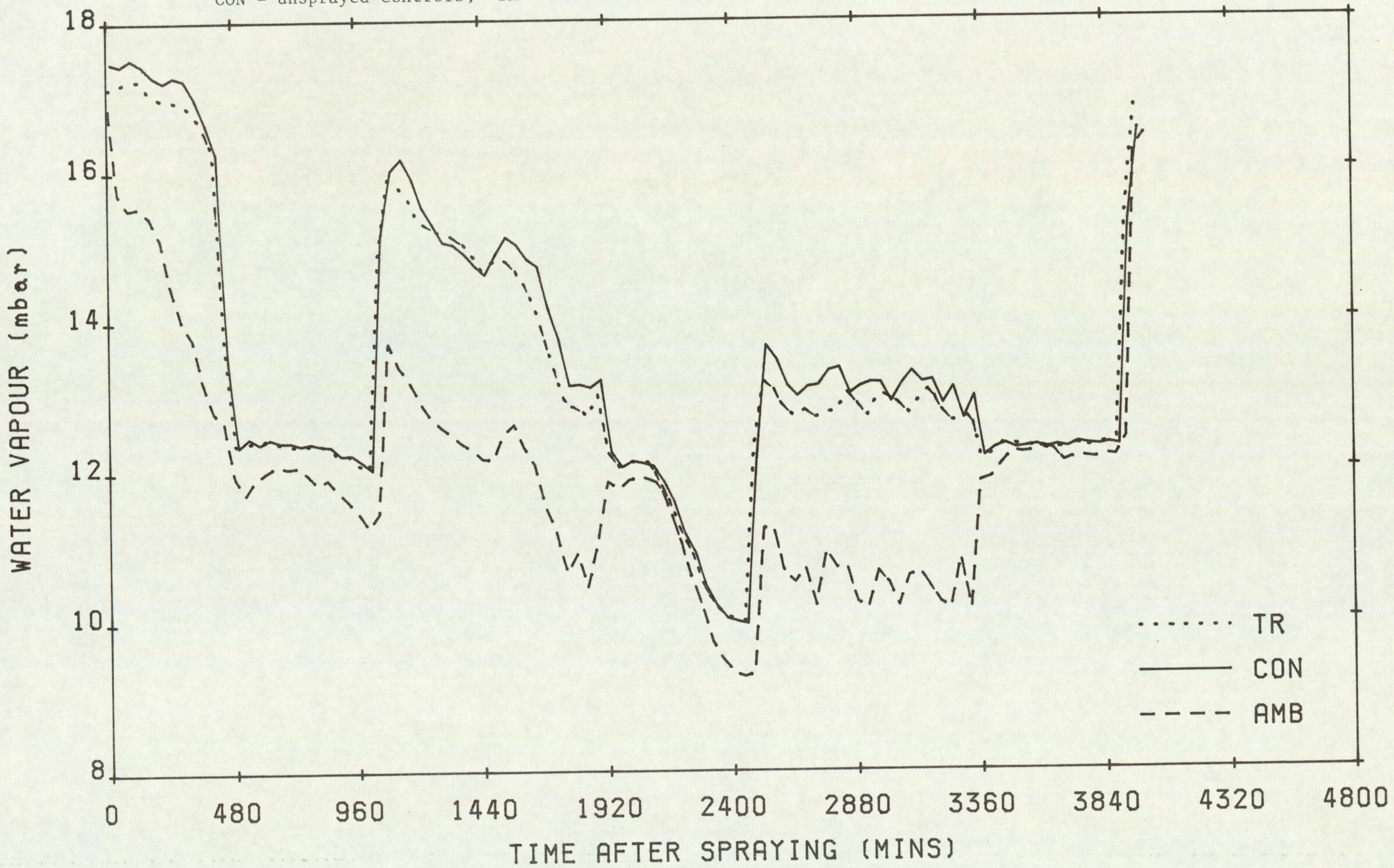


Fig. 6. Water vapour pressure for air leaving cuvettes containing plants of S. media (mean of 6 replicates) CON = unsprayed controls; TR = Plants treated with 0.4 kg/ha ioxynil octanoate; AMB = Ambient water vapour.



ABBREVIATIONS

ångström	Å	freezing point	f.p.
Abstract	Abs.	from summary	F.s.
acid equivalent*	a.e.	gallon	gal
acre	ac	gallons per hour	gal/h
active ingredient*	a.i.	gallons per acre	gal/ac
approximately equal to*	≃	gas liquid chromatography	GLC
aqueous concentrate	a.c.	gramme	g
bibliography	bibl.	hectare	ha
boiling point	b.p.	hectokilogram	hkg
bushel	bu	high volume	HV
centigrade	C	horse power	hp
centimetre*	cm	hour	h
concentrated	concd	hundredweight*	cwt
concentration	concn	hydrogen ion concentration*	pH
concentration x time product	ct	inch	in.
concentration required to kill 50% test animals	LC50	infra red	i.r.
cubic centimetre*	cm^3	kilogramme	kg
cubic foot*	ft^3	kilo ($x10^3$)	k
cubic inch*	in^3	less than	<
cubic metre*	m^3	litre	l.
cubic yard*	yd^3	low volume	LV
cultivar(s)	cv.	maximum	max.
curie*	Ci	median lethal dose	LD50
degree Celsius*	^oC	medium volume	MV
degree centigrade	^oC	melting point	m.p.
degree Fahrenheit*	^oF	metre	m
diameter	diam.	micro ($x10^{-6}$)	μ
diameter at breast height	d.b.h.	microgramme*	μg
divided by*	÷ or /	micromicro (pico: $x10^{-12}$)*	μμ
dry matter	d.m.	micrometre (micron)*	μm (or μ)
emulsifiable concentrate	e.c.	micron (micrometre)*†	μm (or μ)
equal to*	=	miles per hour*	mile/h
fluid	fl.	milli ($x10^{-3}$)	m
foot	ft	milliequivalent*	m.equiv.
		milligramme	mg
		millilitre	ml

† The name micrometre is preferred to micron and μm is preferred to μ.

millimetre*	mm
millimicro* (nano: $x10^{-9}$)	n or mμ
minimum	min.
minus	-
minute	min
molar concentration*	M (small cap)
molecule, molecular	mol.
more than	>
multiplied by*	x
normal concentration*	N (small cap)
not dated	n.d.
oil miscible concentrate	o.m.c. (tables only)
organic matter	o.m.
ounce	oz
ounces per gallon	oz/gal
page	p.
pages	pp.
parts per million	ppm
parts per million by volume	ppmv
parts per million by weight	ppmw
percent(age)	%
pico (micromicro: $x10^{-12}$)	p or μμ
pint	pint
pints per acre	pints/ac
plus or minus*	±
post-emergence	post-em
pound	lb
pound per acre*	lb/ac
pounds per minute	lb/min
pound per square inch*	lb/in^2
powder for dry application	p. (tables only)
power take off	p.t.o.
precipitate (noun)	ppt.
pre-emergence	pre-em.
quart	quart
relative humidity	r.h.
revolution per minute*	rev/min
second	s
soluble concentrate	s.c.
soluble powder	s.p.
solution	soln
species (singular)	sp.
species (plural)	spp.
specific gravity	sp. gr.
square foot*	ft^2
square inch	in^2
square metre*	m^2
square root of*	$\sqrt{\ }$
sub-species*	ssp.
summary	s.
temperature	temp.
ton	ton
tonne	t
ultra-low volume	ULV
ultra violet	u.v.
vapour density	v.d.
vapour pressure	v.p.
varietas	var.
volt	V
volume	vol.
volume per volume	v/v
water soluble powder	w.s.p. (tables only)
watt	W
weight	wt
weight per volume*	w/v
weight per weight*	w/w
wettable powder	w.p.
yard	yd
yards per minute	yd/min

* Those marked * should normally be used in the text as well as in tables etc.

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26. The post-emergence selectivity of some recently developed herbicides: bentazon, EMD-IT 6412, cyprazine, metribuzin, chlornitrofen, glyphosate, MC 4379, chlorfenprop-methyl. October 1973. W G Richardson and M L Dean. Price - £3.31

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