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MEASUREMENTS OF PLANT CARBON DIOXIDE EXCHANGE BY INFRA-RED ABSORPTION UNDER CONTROLLED CONDITIONS AND IN THE FIELD[1]

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In terrestrial plants, photosynthesis and respiration are determined most conveniently by measurement of carbon dioxide exchange. The most accurate and sensitive technique of measuring CO_2 concentration in air is infra-red absorption. Infra-red gas analyzers have been described in detail in several places, e.g. Huber (1958). Such instruments, which require a power supply, can detect differences in CO_2 concentration of the order of one part per million and can be used in the laboratory or in the field.

A. Measurements of CO_2 Exchange Under Controlled Conditions

Measurements of this kind are valuable to study the precise effect of one or more external or internal factors on photosynthetic or respiratory rates. Such measurements have been made by many workers, among them Pisek, Winkler and Larcher in their determinations of photosynthetic capacity ("Assimilationsvermögen"), as reported by Winkler in this Symposium.

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Open, closed, and mixed systems

The chambers used for measurement of CO_2 exchange can be part of open, closed or mixed systems. In an open system air is passed over the plant at a constant flow rate, through the analyzer, and released. The product of flow rate by the difference in the CO_2 concentration of the air before and after passing through the chamber gives the rate of CO_2 exchange of the enclosed material.

In a closed system, air is recirculated in an enclosed volume including the chamber and the sampling tube of the analyzer. The rate of change of CO_2 concentration within the airtight system is a measure of the intensity of CO_2 exchange by the enclosed material.

The several advantages of open over closed systems are that:

1. The plant material is exposed to a relatively constant CO_2 concentration, equal on the average to the mean of the CO_2 concentrations of incoming and outgoing air. The latter can be controlled by manipulations of flow rate. In a closed system the CO_2 concentration varies continuously and, because of the effect of this variation on photosynthesis, it is necessary to make the measurements within a small range of concentration often set at ±10% of the mean value (Decker 1947).

2. In the open system the plant material is usually exposed to a constant relative humidity which can be controlled by hydrating or dehydrating incoming air. In the closed system, relative humidity increases as a result of transpiration and may interfere with CO_2 analysis by producing a signal in the detector, since water vapor shares part of its infra-red absorption spectrum with CO_2.

3. Temperature control is more easily achieved in open systems especially at extreme values.

4. Only a sample of air needs to be passed through the analyzer tube so that a) leaks in the system are not as critical as in closed systems, where they make the readings meaningless; b) several chambers can be used simultaneously and sampled in sequence.

5. Open systems lend themselves to the use of a flowing reference in which the reference tube of the analyzer is filled with a flowing sample of incoming air, which allows higher sensitivity.

On the other hand, closed systems have the following advantages:

1. Measurements can often be made more rapidly.

2. Flow rate control is not critical whereas it is essential in open system measurements.

3. Air velocity can be increased to any value, which may be useful in studying the effect of wind on CO_2 exchange.

Mixed systems have some of the advantages, and disadvantages, of open and closed systems. Most of the air is recirculated while a small fraction is constantly renewed. One such elaborate system is used at the "Klimabiologischen Versuchsanstalt" at Patscherkofel, near Innsbruck, Austria.

The choice of the system to use must be guided by the purpose of the investigation.

Light Sources

A number of light sources have been used for measurements under controlled conditions: incandescent bulbs, fluorescent tubes, mercury high-pressure lamps, high-pressure xenon lamps (Rüsch and Müller 1957). We have used a "Flucmeric" lamp which combines an arc with incandescent filaments and phosphors. This lamp gives a fairly well-distributed spectrum and light intensities of over 120,000 lux are easily obtained with the 1700-watt model.

Whatever the light source, consideration should be given to the importance of expressing light quantities in units of visible energy. A comparison between the energy values per lux of various commonly used light sources was made by Gaastra (1959). He found that the number of ergs cm^{-2} sec^{-1} per lux varied among them from 2.47 to 4.23. Because of this variation and because of differences in emission spectra it is often difficult to compare photosynthetic rates obtained by different authors. It might be desirable to have all workers in this field agree to use the same type of lamp or at least to express light in energy units.

Chambers

Chambers are made to fit the plant or plant part to be investigated. They can be very simple if measurements are made at room temperature and if rigorous temperature control is not necessary. When the latter is critical, it can be achieved by using "Plexiglas" chambers fitted with upper and lower jackets in which the circulation of water or coolant is controlled by a relay activated by a temperature sensing device placed in the chamber. With alcohol-water mixtures as coolant a temperature of 0°C at a light intensity in excess of 80,000 lux can be maintained easily. A layer of running water several cm deep is usually placed between the lamp and the chamber to act as a heat filter. Temperature control can also be effected by including in a closed system the cooling coils of a small air conditioner.

In the design of chambers consideration must also be given to the positioning of the leaf and to the pattern of air movement.

Humidity control

Humidity control can be achieved by bubbling the air through columns of water or mixtures of sulphuric acid and water.

Control of the CO_2 concentration

CO_2 concentration can be adjusted by mixing CO_2-free supply air with pure or diluted CO_2. For concentrations substantially above normal it is necessary to use a flowing reference in order to maintain high sensitivity. In this fashion good sensitivity can be obtained at concentrations up to 0.2% CO_2.

Attached versus excised branches or leaves

The effect of severing of branch or leaf on the photosynthetic rate is the object of controversy. Clark (1954) found that there was no change in the photosynthetic rates of spruce branches for about 90 minutes after cutting. We arrived at the same conclusion with regard to short shoots of spruce, hemlock, and pines. Pisek and Winkler (1958) and Larcher (1960) found that they could validly measure photosynthetic capacity ("Assimilationsvermögen") on cut twigs as long as the water deficit remained small. Koch and Keller (1961) observed decreases in photosynthetic rate after cutting of poplar leaves under water, and increases after cutting in air. They concluded that cut leaves should not be used for ecological investigations. Brun (1961) found that interrupting the vascular supply to young banana leaves resulted in a temporary increase in the rates of photosynthesis. Mortimer (1959) stated that the rates of assimilation by freshly excised leaves of barley, bean, cabbage, cotton, geranium, sugar beet, and tobacco were equivalent to those of leaves still attached to the plant. Cursory observations we have made on excised needle fascicles of Pinus rigida, maintained in a moist atmosphere, indicate that steady rates of photosynthesis prevail for as long as one hour. After that time rates decline steadily, probably as a result of water loss.

Whenever feasible we use attached leaves or branches, the petioles or bases of which extend through slots in one of the chamber walls. Care has to be exercised in sealing to avoid leaks and damage to plant tissue.

Induction period

As Clark (1954) and others have observed photosynthetic rates may not reach a steady level for several minutes (15 in Picea abies) after illumination has started. To avoid this effect, which is presumably due to induction phenomena as well as to stomatal movements, we routinely expose the plant to a light source similar to that used in the measurements for at least 1/2 hour before these measurements are made.

Calibration of the infra-red analyser

The most convenient procedure for calibration of the analyzer consists in using a set of standard mixtures of CO_2 in nitrogen. The instrument can also be calibrated by injecting known amounts of gaseous CO_2 into a closed system by means of a hypodermic syringe or by placing inside the system a known weight of dry ice, or by generating CO_2 from a carbonate solution. Release of known amounts of CO_2 can also be used to measure accurately the volume of a closed system.

Combination of infra-red and C^{14} techniques

An apparatus combining infra-red absorption and use of carbon-14 has been devised by Lister, Krotkov and Nelson (1961). It consists of a closed system in which the concentration of total CO_2 is monitored with an infra-red gas analyzer and that of $C^{14}O_2$ with a Geiger counter. When the CO_2 compensation point is reached in light the continued decrease in specific activity of $C^{14}O_2$ in the system is an indication of the amount of CO_2 released by the tissue, i.e. light respiration, which can be compared with dark respiration, measured by increase in total CO_2 concentration in the system.

B. Field Measurements

Line current is the most convenient and reliable power source for field measurements with the infra-red gas analyzer, although portable generators and batteries can be used. Measurements of CO_2 exchange in the field can be made in two ways: by enclosing the plant material in a chamber or by determining concentration gradients above the plant cover.

Measurements with chambers

Field measurements of CO_2 exchange are possible using open or closed systems and chambers similar to those used in the laboratory.

Chambers are usually made of a transparent material of a shape and size suited to the type of plant being investigated. Two difficulties exist: first the temperature inside the chamber increases greatly especially in full sunlight because of long-wave radiation trapping. The second is that relative humidity builds up inside the chamber and eventually water condenses on the walls thereby reducing light transmission. These difficulties can be overcome in two ways. The first is to have the chambers air-conditioned as in the very ingenious devices built by Bosian (1959). The other approach is to move air through the chamber at such a rate that heating is reduced to a minimum, relative humidity does not increase and condensation is avoided. A compromise must be reached between the desirability of having high flow rate to prevent excessive heating and the necessity of obtaining a measurable differential in CO_2 concentration.

A technique of this kind has been used by Tranquillini (1959) who enclosed twigs of pines and other conifers in small cylindrical chambers.

A new type of chamber for outdoor use has been devised by Lange (report given at the Innsbruck International Symposium of Tree Physiology, September 1961). Made for single leaves it is fitted with a lid which can be closed or opened by remote control. The lid is maintained in the down position just long enough to obtain a steady reading of CO_2 exchange and then lifted. This prevents heating and increase in relative humidity of the air.

We have attempted to devise an automatic multiple point system to measure CO_2 exchange of whole branches of pine trees in an oak-pine forest subjected to chronic gamma irradiation at Brookhaven National Laboratory, Long Island, New York. A description of this system follows.

The chambers (Fig. 1) are made of 0.008 inch polyvinyl chloride film. This is a clear, tough, pliable material that has a smaller heat trapping effect than glass or "Plexiglas." The chambers are rectangular in cross-section and of a size suitable to enclose a pine branch. Dimensions vary but one typical chamber may be 50 cm x 45 cm in section and 60 cm long. The upper part is stretched on a frame of "Plexiglas" and aluminum rods, whereas the lower half hangs loosely. One of the small sides is open whereas the other consists of a plate of "Plexiglas" perforated at its center to accommodate a 25 cm length of 5 cm diameter "Plexiglas" tubing at the remote end of which a squirrel cage type blower is attached. Inside the chamber at 5 cm from the inlet plate there is a parallel perforated plate serving as a baffle to insure even distribution of air through the chamber. The branch is introduced into the chamber from the opposite side. The chamber is attached to a pole in such a fashion as to leave the branch in as nearly natural a position as possible. Chamber and pole tend to sway in the wind in the same fashion as branch and tree do. A skirt of thinner film extending the chamber is loosely tied around the base of the branch.

Air is sampled at two places, one near the inlet of the blower, the other in a plane normal to the direction of air flow and corresponding to the junction between the chamber proper and the skirt. The air collecting device in that plane consists of two perforated butyrate tubes running diagonally across the chamber and connected together. Thermocouple junctions are placed along the branch and read automatically. Installation of light sensing devices is planned.

Flow rates of the order of several hundred liters per minute can be obtained. Flow adjustment is achieved by varying the voltage supply to the blower or by using blowers of various capacities. Flow rate is determined by measuring air velocity in the tube between blower and chamber by means of a thermoanemometer. Velocity readings may be converted to flow rates using values obtained by calibration with a large capacity Rotameter-type flow meter. Using these flow rates it has been possible, at least through the winter, to limit the increase in temperature in full sunlight to 4-5 degrees centigrade, and to prevent condensation.

Determination of CO_2 differential is made on samples of air drawn from the two sampling points at a rate of 5 to 7 liters per minute by means of a diaphragm pump and directed to the gas analyzer. By using a 12-point "Gelman" sequential valve (Fig. 2) 6 chambers with 12 sampling points can be operated simultaneously. A timer with a 5-minute cycle controls the sequential valve so that one sampling series is completed in one hour. The 5-minute cycle is sufficient to handle a sample originating from the most distant point to the analyzer (130 m).

The air stream is dehydrated by passage over "Drierite" ($CaSO_4$) prior to entering the analyzer. The latter is connected to a Leeds and Northrup recording potentiometer with adjustable zero and adjustable range so that any portion of the analyzer range can be expanded over the width of the strip chart. Reliable measurements of differences in CO_2 concentration of the order of 1 ppm can thus be obtained, which makes it possible to operate at high flow rates. Another recording instrument provides data on temperature ~~and light intensity~~ in the chambers (Fig. 3). Total solar radiation is measured by a recording pyrrheliometer located in an open area close to the trees being investigated.

This apparatus would also lend itself to determination of transpiration by measuring the relative humidity of the air samples with an electric hygrometer. For this measurement, however, condensation inside of the tubing has to be avoided by keeping its temperature above dew point.

The same technique will be applied to measurements of CO_2 exchange by entire shrubs and by the soil, using chambers of appropriate shape and size. It can also be used in measuring respiration by tree stems. For this a segment of the stem is loosely wrapped in a plastic sleeve and air is drawn from a collecting device placed at mid-height inside the sleeve.

It is planned to extend this technique to operate on whole trees which would be enclosed in clear frameless tents inflated by a large capacity blower. The base of the tent would be kept pressed to the ground by means of water or sand bags.

Data obtained in winter on <u>Pinus rigida</u> with this apparatus are presented by Woodwell and Bourdeau in this Symposium.

An essentially similar technique has been used by Moss (1960) to measure photosynthesis of field crops and a somewhat more elaborate portable system by Polster, Weise, and Neuwirth (1960).

<u>Measurement of CO_2 flux</u>

In perfectly still air, gradients of CO_2 concentration above a plant cover can be used to measure CO_2 exchange. During the day CO_2 is depleted at plant level and concentration increases with height whereas the reverse is true at night. However, wind reduces the steepness of the gradients. By combining wind velocity data and CO_2 concentration at 2 or more heights above the plant cover it is possible to measure CO_2 flux, i.e. the net movement of CO_2 from or into the vegetation. This technique was first developed by Huber (1950) and has been refined and used successfully by Monteith and Szeicz (1960) and by Lemon (1960) over agricultural crops. It has apparently not been applied to forest stands.

C. Conclusions

Infra-red gas analysis provides the most rapid and accurate method of measuring CO_2 exchange by terrestrial plants in the field or in the laboratory. The main drawbacks are the necessity of a power supply and the bulk and cost of the equipment. A compact, easily portable analyser with reduced power requirement would be extremely useful.

Measurements of CO_2 exchange are especially valuable in providing rapid estimates of productivity of ecosystems. They are also useful in studying the response of plants to environmental and genetic factors, or to various cultural treatments.

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Figure Legends

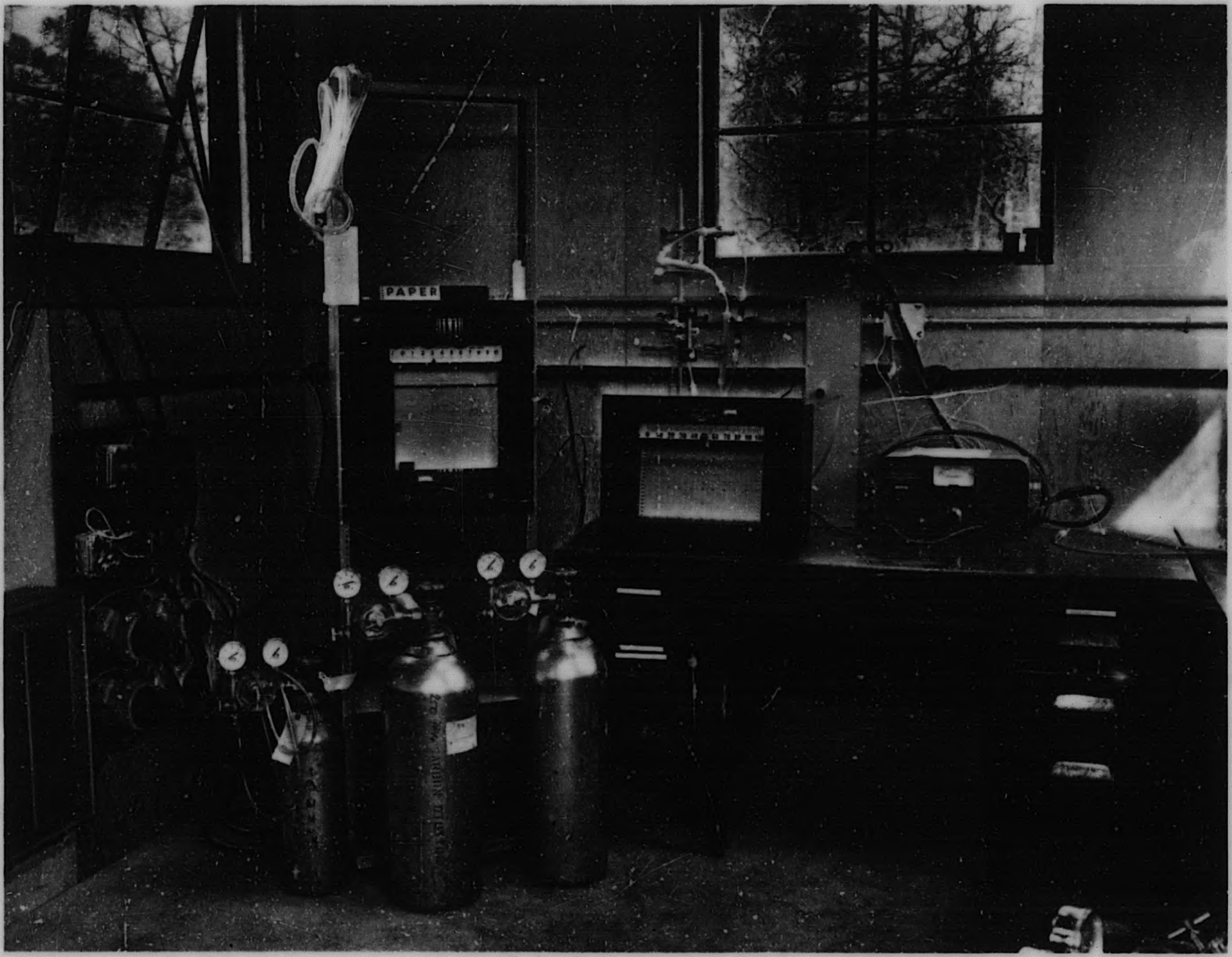
Fig. 1. A chamber used for field measurement of plant CO_2-exchange. Air, supplied by the blower at rates up to several hundred liters per minute, passes over the branch and is sampled at the open, exhaust end of the chamber. The arrow designates one of the two sampling tubes. Input air is sampled through the tube adjacent to the blower.

Fig. 2. The 12-point sequential sampling valve and 5-minute-cycle clock mounted for field use.

Fig. 3. Infra-red gas analyzer and auxiliary equipment mounted for field use. Variable transformers control blower voltages. Recorder on the left records analyzer signal; on the right, thermocouple signals from within and without chambers.

PLEASE REFER TO BNL NEG.# 2-113-62. FIG.#1

PLEASE REFER TO BNL NEG. #9-888-62, FIG #2



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