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PRESSURE CHAMBER AND AIR FLOW POROMETER FOR RAPID FIELD INDICATION OF WATER STATUS AND STOMATAL CONDITION IN WHEAT

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SUMMARY

Plant water potential and leaf diffusive conductance, key features of plant responses to water stress in field experiments, can be estimated, respectively, by xylem pressure potential measured with the pressure chamber apparatus, and leaf permeability measured with the air flow porometer. This paper describes modifications to these two techniques in order to increase the rapidity of measurements in wheat to 60/h with the pressure chamber, and 200/h with the porometer. Rapid measurements are needed because of the large within- and between-plot errors encountered with daytime measurements in typical field experiments, examples of which are presented.

Leaf water potential is a key to the water status of plants. In comparisons between cultivars under dry conditions it could provide a useful indirect measure of water economy or extent of the root system. Similarly leaf diffusive conductance, governed largely by stomatal aperture, is a major controlling factor in leaf photosynthesis, and specially in transpiration. As a result, indirect relations have been proposed between stomatal opening and yield, and stomatal closing and drought resistance. However, there have been few attempts to use measurements of leaf water potential or leaf diffusive conductance in comparing cultivars in the field, with a view to their ultimate use as criteria in breeding programmes. Variability found in field studies, especially under dry conditions; the number of cultivars which it is desirable to study; and the limited time throughout the day during which values might be stable; all demand a rapidity of measurements unattainable with present instruments.

Leaf water potential can be estimated by xylem pressure potential, measured with the pressure chamber (Ritchie and Hinckley, 1975), and leaf diffusive conductance for amphistomatous leaves by measuring leaf permeability with a viscous air flow porometer (Meidner and Mansfield, 1968), both of which are suitable for relatively rapid measurements in the field. This study describes modification of these instruments to speed up and simplify collection of field data on cultivars of wheat (*Triticum aestivum* L.).

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METHODS

Pressure chamber

A commercial pressure chamber (PMS Instrument Co., Corvallis, Oregon) was used, in which the pressure seal comprised a tapered rubber stopper fitted into a tapered hole in the metal chamber top. Both leaves and stems could be sealed into the chamber. For leaf measurements, lamina were torn off close to the stem, and usually two lamina were collected and measured simultaneously. However, since most leaves were too wide to pass through the pressure seal, part of the lamina at the base of the sample was torn back parallel to the midrib, leaving a strip of lamina about 5 mm wide and long enough to pass through the pressure seal (a standard No. 5 rubber stopper, slit to about three-quarters of its diameter) and the slit-like opening in the metal cap of the chamber, and to protrude sufficiently for observation. Severing the leaf lamina in the rubber stopper under pressure was prevented by bevelling the down-pressure edges of the slit in the stopper.

Various pressure chamber measurements on stems of wheat were used. In initial studies samples were cut 5–10 cm below the top prominent stem node, inserting all the upper part of the shoot into the chamber. The basal end of the shoot sample was passed through a hole in the rubber stopper and metal cap, locating the stem node just against the up-pressure side of the stopper, to achieve a very satisfactory pressure seal. A thin-walled copper tube was used to expand the hole in the rubber stopper, thereby facilitating the loading of each sample. The measurement obtained is referred to as the *shoot xylem pressure potential.*

A modification involved measurements on spikes alone, making the pressure seal just below the beginning of the rachis. In part these two techniques were adopted to conform to the methodology outlined by the developers of the pressure chamber (Scholander *et al.*, 1965), but a third technique was later found more satisfactory. Samples were obtained by cutting 10–15 cm above the uppermost stem node immediately after making a similar cut below the node. The sample was thus reduced to a stem segment, covered by leaf sheath in the above-node portion, giving results referred to as *stem xylem pressure potential.* Samples were placed in the pressure seal in the same way as the whole shoots, but the reduction in size of sample permitted the collocation of six samples in the pressure chamber. Six holes were cut in the rubber stopper with a cork borer and six matching holes were drilled in the metal cap (after suitable testing to establish that the cap was not dangerously weakened). The holes in the stopper and cap were numbered so that each sample could be identified.

The use of a 10 cm magnifying glass facilitated the observation of end points when either two or six samples were being measured simultaneously. As the end-points were approached, the pressure was increased at about 0·3 bar/s. Upon the appearance of an end point, pressure build-up was stopped and the pressure recorded for the particular sample; the pressure increase was then resumed. In this manner unclear end-points, associated with bubbling at the cut surface, could usually be checked as the pressure rose further. Often the sample showed a second

more obvious end-point, which was taken to be the true value. The small sample size with leaves and stem segments permitted a considerable reduction in pressure chamber volume and hence conservation of compressed gas. The use, on the pressure line to and from the chamber, of taps having only a quarter turn from open to closed also facilitated rapid measurements.

Because of continuing water loss through transpiration all samples were immediately placed in a shaded humid plastic bag as they were collected and kept there as far as possible during loading of the pressure seal, only to be removed just before the stopper was inserted into the metal cap and the cap fitted to the chamber for the actual measurement. A moistened cloth pad was kept in the bottom of the chamber at all times.

Air flow porometer

The instrument used, commonly known as the Alvim porometer (Meidner and Mansfield), consisted basically of the plexiglass pistol described in Figure 3.1 of Hsiao and Fischer (1975). To this pistol, which contained the porometer cup (7·5 mm neoprene O-rings), was attached the manometer (Minihelic Gauge for 0–5 in water; Dwyer Instruments, Michigan City, Illinois), the sphygmomanometer bulb for pressurizing the system, an air reservoir (small plastic bottle) and connecting tubing. The low pressures (< 5 in water) used in this system should have avoided all possibility of leaf distortion and change of aperture in response to the pressure gradient applied. The bulb was attached to the pistol butt so that the system could be pressurized by the same hand which held the porometer attached to the leaf. With experience, a single squeeze of the bulb was sufficient to achieve the required starting pressure of 4·5–5 in of water. There were no taps in the system, the operator simply noting the pressure that was reached 10 s after the falling manometer needle indicated 4·0 in of water. Using a stopwatch, and after some practice, this operation could be carried out with little error. Pressure on the O-rings of the porometer cup, maintained with elastic bands, was sufficient to cause some tissue damage. Lighter pressure gave similar permeabilities but the heavy pressure was preferred to eliminate any possibility of leakage, while the annulus of necrotic tissue usefully marked the positions that had been sampled. Air flow was always from the abaxial to adaxial surface.

Calibration of the porometer, to give leaf permeability in cm^2 s/g, was carried out with a glass capillary of known diameter, whose resistance to air flow was calculated from Poiseulle's law. Results were expressed in convenient arbitrary units, namely the square root of (cgs permeability $\times 10^3$). With the porometer as described (0·44 cm^2 cup area), an internal volume (plastic bottle plus manometer plus tubing) of 940 cm^3 gave convenient 10 s pressure drops when stomata were fully open; a second, smaller, volume (670 cm^3) was used under less favourable conditions. The air flow porometer was calibrated against leaf diffusive conductance (cm/s) to water vapour measured with a diffusive resistance porometer of the hygrometer type (Cayuga Development Corporation, Ithaca, NY).

EXPERIMENTAL MATERIAL

Testing was conducted during the 1973–74 and 1974–75 winter cropping seasons at the Centro de Investigaciones Agricolas del Noroeste (CIANO), near Ciudad Obregon in north-west Mexico. Wheat cultivars were grown under irrigation and with optimal agronomy in plots from 4·5 to 100 m^2 in size, arranged in replicated trials. Trials were sown during November–December and headed in about early March. Measurements of xylem pressure potential and leaf permeability were made between February and April, under mostly clear skies, with daily maximum air temperatures fluctuating between 20 and 35°C and maximum vapour pressure deficits between 10 and 40 mbar. Soil drought was created by withholding irrigations.

The relations between xylem pressure potentials, determined for different parts of the wheat plant, were studied by measurements on matched shoots in the field during daytime. Measurements on a number of cultivars during the daytime period of stable minimal values, approximately 2 h before to 4 h after solar noon (Sojka, 1974), provided estimates of the variability of xylem potential on a number of occasions. Shoots were chosen at random from the plots, but regions within 50 cm of the plot edge were avoided.

Measurements of leaf permeability were made in the middle of the uppermost fully-expanded and sunlit leaves, chosen otherwise at random from within the canopy. Leaf permeability was calibrated against leaf diffusive conductance on one day when a range of water stress levels, obtained in various experiments, induced a range of permeabilities. Diurnal changes in permeability were studied on several occasions in order to determine when permeability was most stable. Measurements in a number of replicated trials, during the mid-day period of relatively stable permeability, enabled calculation of the error in leaf permeability due to within and between plot variability.

RESULTS AND DISCUSSION

Pressure chamber

After heading, spike xylem pressure potentials were always lower (more negative) than shoot potentials, with an average difference of 1·0 bar over a wide range of situations. Xylem pressure potential of the flag leaf was always within 2 bar of spike potential, tending to be lower than the latter under conditions when one would expect rapid transpiration. The pressure potential of stem segments was consistently higher than shoot potential when stress was minimal, but tended to approach shoot potentials at more severe stress levels (Fig. 1a). The relation of stem potential to flag leaf potential was also good, with the former higher than the latter by from 1 to 7 bar (Fig. 1b).

The small consistent differences between the potentials described above are in the direction, and of the magnitude, to be expected from gradients caused by transpirational water flow (Meiri *et al.*, 1975; Millar and Denmead, 1976). However, it is surprising that stem segments gave apparently valid xylem pressure potential values. Much higher values may have been expected as a result of the

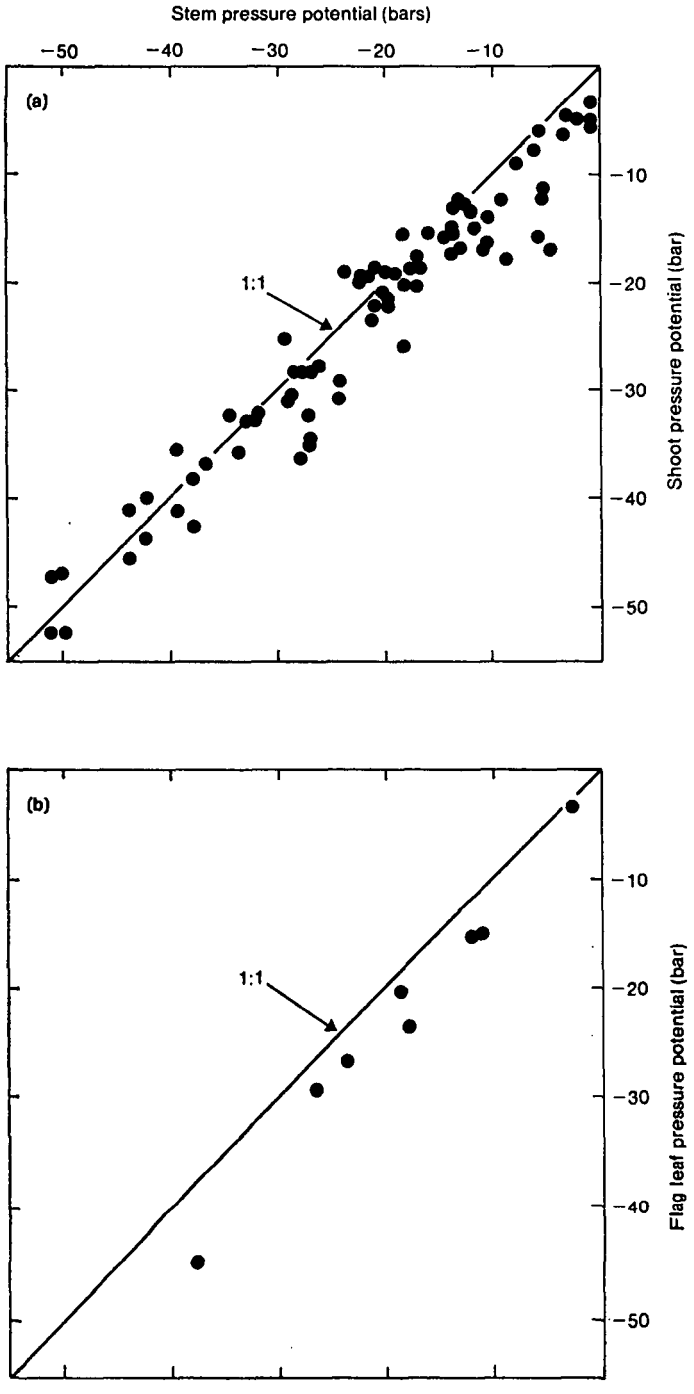

Fig. 1. (a) Relation of shoot xylem pressure potential to xylem pressure potential of stem segments; pairs of matched shoots on numerous occasions, 1973–74. (b) Relation of xylem pressure potential of flag leaves to that of stem segments; groups of 12 pairs of matched shoots on each of several occasions in March and April 1975.

cut, within the pressure chamber, in the xylem of the sample, since the diameters of xylem vessels are not sufficiently small to offer any resistance to air entry (even 2 μm diameter vessels would drain at a pressure differential of 1·5 bar). The explanation may lie in the observed replacement of xylem vessels by smaller diameter tracheids in the nodal regions of wheat (Percival, 1921), and/or the presence of crosswalls in the xylem vessels in internodal regions. Another aspect is that substantial trimming of a shoot sample by a second cut before the sample is inserted into the pressure chamber (for example cutting at ground level, then cutting 5–10 cm below the uppermost stem node) appears to increase the equilibrium pressure potential measured only slightly (<2 bar). Scholander *et al.* advise against trimming, except where xylem element crosswalls prevent retraction of the xylem water for any distance from the cuts, which appears to be the case (according to them) for conifers and monocotyledons.

In the multiple sample methods, the time between removal of a sample from the plant and its measurement in the pressure chamber increases. However, by storing in a humidified plastic bag in the shade, the pressure potential of leaf samples over this period declined at only about 0·2 bar/min, while that of stem segments did not change at all for at least 10 min. Depending on the distance between the pressure chamber and the plot being sampled, and upon stress levels, samples were thus stored for up to 5 min in a system employing two persons, one sampling and one measuring, with measurement rates up to 60/h for leaves and somewhat greater for stem segments. End points were generally easier to see on leaves, but with post-anthesis stress, leaf tissue senesces well before stem tissue, and cannot therefore be used throughout.

The importance of being able to make rapid measurements is seen from the variability of field data. Repeated sampling in the central part of given small plots (4 m^2) during daytime gave a standard deviation per individual sample of about 1·5 bar for both leaves and stem segments under well-watered conditions (xylem pressure potentials > -20 bar). However, under stress (potentials < -30 bar), this figure rose to around 5 bar. Thus a standard error of the plot mean of less than 1 bar would require from 2 to 25 samples per plot, depending on the stress level. Between-plot errors further increase this number, in experiments comparing a number of treatments or cultivars. For example, sampling two leaves per plot for each of 54 cultivars, in a randomized block experiment with three replications and plots of 4·5 m^2, gave a standard error of cultivar mean ranging from 0·9 to as high as 3·0 bar, increasing generally as the level of soil water stress increased.

A number of possible sources of error with the pressure chamber were not studied here, including the exclusion error, which appears to depend on the relative amount of the sample outside the chamber (Millar and Hansen, 1975), the assumption that the solute potential of the xylem sap can be neglected, and water loss from the tissue when it is inside the pressure chamber (Baughn and Tanner, 1976). Attempts to calibrate the technique against total water potential have met with good success in many species (Ritchie and Hinckley) but varied

success in the case of wheat (Millar and Hansen): however, some of the problems may actually lie with the measurement of total water potential. Moreover, there has yet to appear a detailed analysis of the effect of water potential gradients within organs that are transpiring rapidly when sampled, except for the study of Meiri *et al.*, who concluded that the pressure chamber measures the highest potential value in such cases. However, in spite of the likelihood of more refinements to the pressure chamber technique, it is concluded that (as presently understood) it is a very useful method for the rapid quantification of plant water stress in many field situations.

Air flow porometer

The results of calibrating the air flow porometer against the diffusion porometer are shown in Figure 2, where each point is the result of four permeability readings on the leaf and one diffusive conductance determination on each surface of the same leaf. The relation of the lowest diffusive conductance (regardless of surface) to leaf permeability (Fig. 2a) is somewhat better than that of abaxial or adaxial conductance to permeability (not shown). This is to be expected, since the air flow porometer measures the permeability of both surfaces in series, and hence is most influenced by the surface of greatest resistance to viscous flow (and presumably lowest diffusive conductance). This is usually, but not always, the abaxial surface in the case of wheat (see also Fig. 2c). The downwards curvature of the relations in Figures 2a and b suggests that the square root transformation of viscous flow permeability was not the most appropriate. The plot of log leaf diffusive conductance against log untransformed permeability gave a linear slope of 0·33 ($r^2=0{\cdot}86$), suggesting the cube root transformation of permeability for linear relations with leaf conductance. Meidner and Mansfield recommend a cube root transformation for graminaceous stomata.

For predictions of possible transpiration or photosynthesis rates, the total diffusive conductance of the leaf (adaxial + abaxial conductances) is the most appropriate, and this was reasonably well predicted from leaf permeability (Fig. 2b). It has been shown with other species that abaxial and adaxial stomata can differ in their response to certain environmental factors, and to time of day; this may lead to a poor relation between leaf diffusive conductance and leaf permeability (e.g. Turner, 1970). The good relation found in Figure 2 suggests there was no such differential stomatal behaviour with wheat, at least when these two cultivars responded to water stress, which was the major cause of variation in this work. Figure 2c confirms that adaxial (Y) and abaxial (X) diffusive conductances were reasonably closely related ($Y=0{\cdot}19+0{\cdot}92X$, $r^2=0{\cdot}59$). The tendency for adaxial to exceed abaxial conductance was also seen by Sojka, who worked in the same environment. Highly significant positive rank correlations between leaf permeability and leaf photosynthesis, found across a set of ten wheat cultivars by Shimshi and Ephrat (1975), support the relation in Figure 2b. Moreover, the sensitivity of the air flow porometer to the surface of lowest conductance could be an advantage in that Rawson *et al.* (1976) have shown in

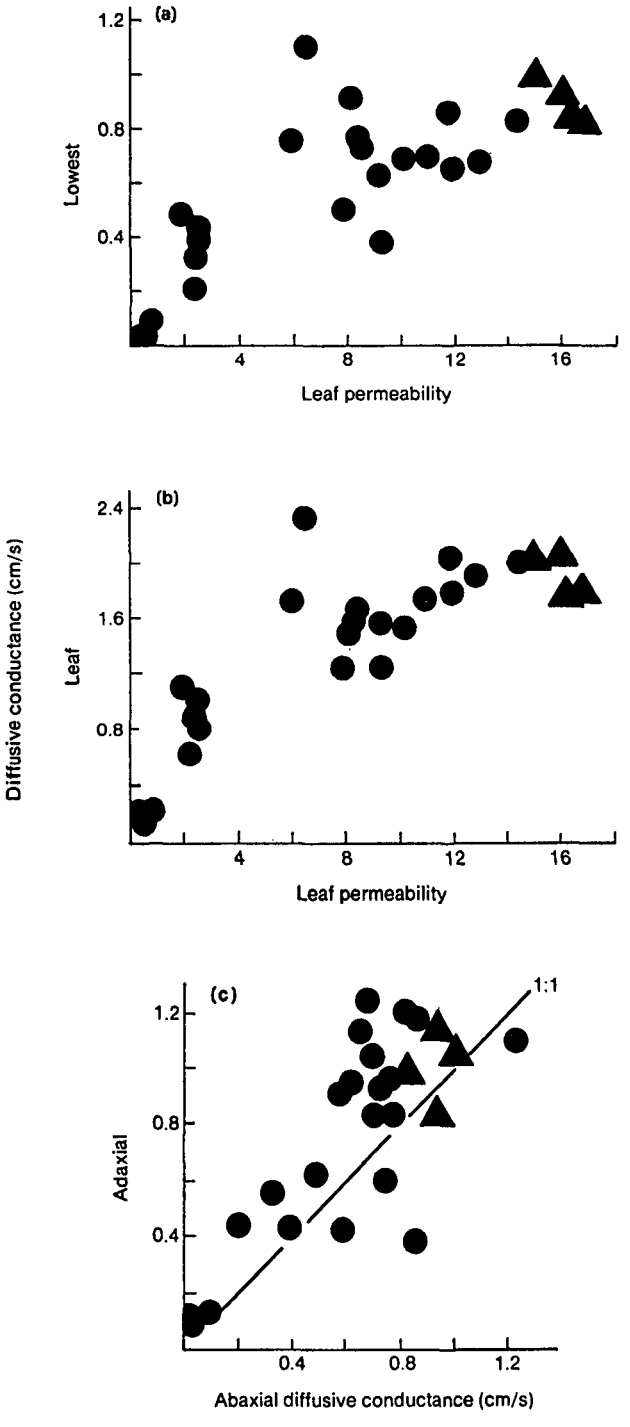

Fig. 2. Relation of (a) lowest diffusive conductance (abaxial or adaxial) to leaf permeability, (b) leaf diffusive conductance (abaxial + adaxial conductance) to leaf permeability, and (c) adaxial and abaxial diffusive conductances; data from 26 attached leaves taken from various field experiments between 1100 and 1230 h on 16 March 1975 (see text); cultivars Yecora 70 (circles) and Fiserect 4A (triangles).

wheat that changes in conductance of the abaxial surface, clearly the surface of lowest conductance in their study, bore the closest relation to variation in leaf photosynthesis induced by source-sink manipulation.

Typical diurnal changes in leaf permeability (Fig. 3) showed little evidence for mid-day closing of stomata, and stomatal opening was apparently relatively stable from about 3 h after sunrise until 2 h before sunset, a period during which measurements designed to compare cultivars or agronomic treatments would be best concentrated. Using a diffusion porometer, Sojka reached a similar conclusion. The apparent temporal fluctuations in permeability for the cultivar Olesen on the morning of 24 February 1975 were not statistically significant, and reflect a significantly higher error variance for that cultivar compared to Yecora 70 measured on the same day.

The variability encountered in collecting the data of Figure 3 was considerable. Pooling the data for each cultivar showed that the standard deviation of a single leaf permeability measurement within a given small plot on the 24 or 25 February ranged from 2·1 to 3·3. Standard deviations were less on the 8 April (0·5 and 1·3), no doubt a result of the lower mean values, because coefficients of variability were higher, averaging around 50%. The standard error of the points shown for 10 March (means of 6×2 leaves) was 0·4.

With large field experiments, between-plot error overrides within-plot error. In a randomized block experiment, comprising 48 cultivars in small plots (10 m^2) under well-watered conditions, with five replicate blocks and three permeability measurements/plot, the standard errors of the cultivar mean varied from 0·5 to 1·0 on different occasions throughout the season. As mean permeability was usually high (>5), such a sampling intensity was sufficient to detect highly significant cultivar effects on leaf permeability on most occasions. Similar experiments under water stress conditions showed lower error variances, but also lower mean leaf permeabilities. For example, two permeability measurements per plot (4·5 m^2) in the randomized block experiment, with three replications and 54 cultivars, produced standard errors of the cultivar mean which ranged from 0·4 to 0·7. However mean permeabilities were always less than five, and significant cultivar effects were usually not detected. Finally, in an agronomic experiment with four replications of irrigation treatments and large plots (100 m^2) of a single cultivar, eight leaf permeability measurements per plot led to a standard error of the treatment mean ranging from 0·2 to 0·8. The effects of differences of one week in time from the last irrigation were usually shown to be significant with this sampling intensity.

The above results suggest a minimum of ten readings (e.g. two in each of five plots) in order to detect useful differences, with a larger number for comparisons under conditions of water stress. These conditions, together with the possibility of accelerated stomatal changes during the course of the day under less stable weather conditions than those experienced here, make rapid measurements imperative. Using this air flow porometer, with a tape recorder to record the results, one person can make 200 measurements/h. The adoption of a constant 10 s pressure drop time

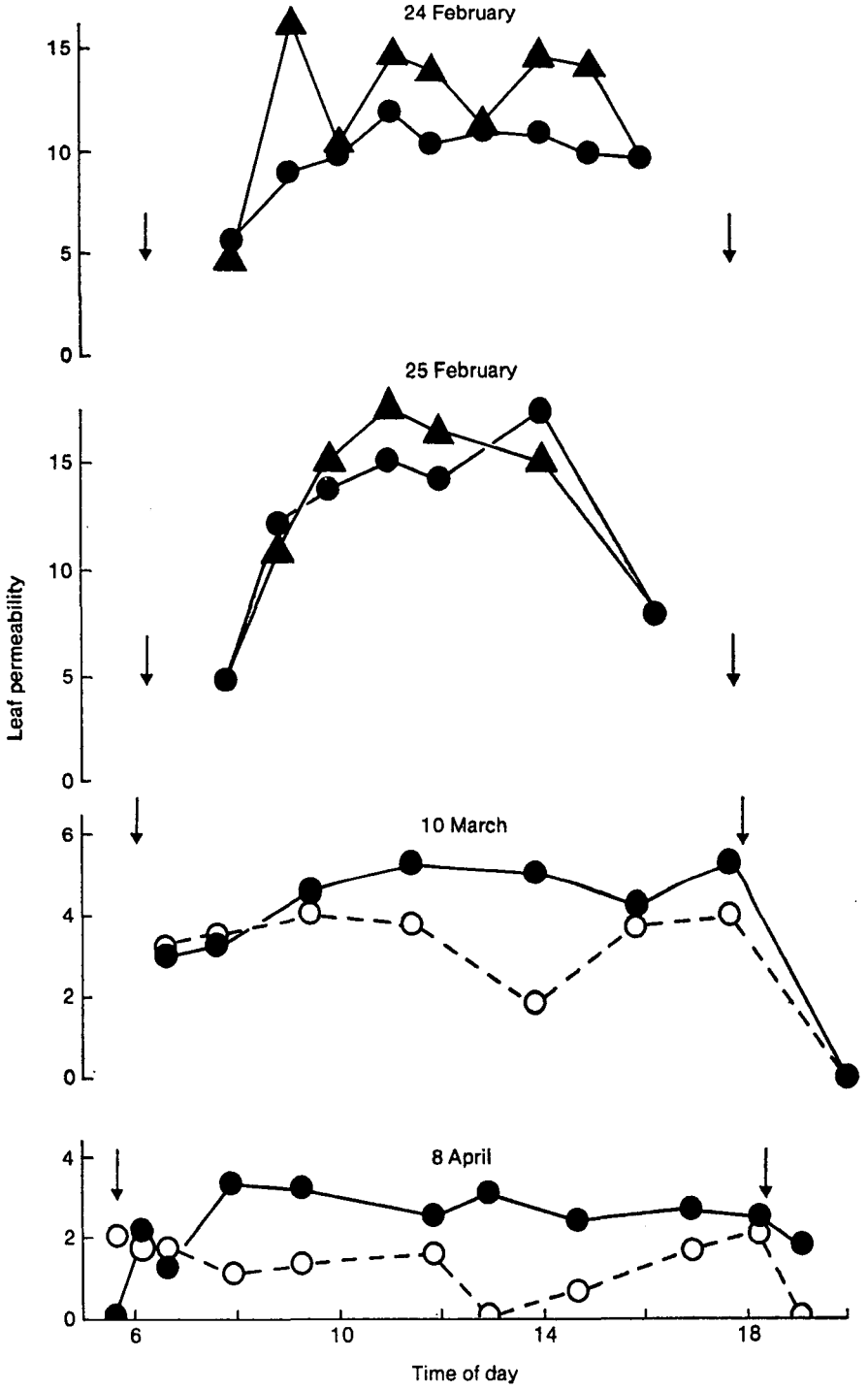

Fig. 3. Diurnal changes in leaf permeability: 24 February 1975, Yecora 70 (●), Olesen (▲); 25 February 1975, Jupateco 73 (●), Tom Thumb (▲); 10 March 1975, Yecora 70 non-stressed (●), Yecora 70 stressed (○); 8 April 1975, T64-2-W (●), T64-2-W stressed (○). Results (except for 10 March) are means of six leaves chosen at random at each point in time from within one small plot of each cultivar; on 10 March, six leaves were taken from each of two plots for each stress level. Except for 10 March and T64-2-W stressed, all plots had been recently irrigated; on 10 March Yecora 70 non-stressed had been last watered 18 days ago, and Yecora 70 stressed 42 day ago; cloudless conditions prevailed; arrows indicate time of sunrise and sunset. See text for statistical analysis.

limits the precision of an individual reading, but this loss of precision is more than adequately compensated by the gain in rapidity of measurement. Downey *et al.* (1972) point to the suitability of the air flow porometer for field studies on similar grounds, and describe a porometer with which one person can make at least 120 measurements/h. Our porometer appears to be both simpler and even faster than Downey's and is recommended for field studies with wheat and related species. Results of its use in cultivar comparisons, under drought and non-drought conditions, and in irrigation frequency experiments, will be presented elsewhere.

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