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A COMPARISON OF DEWPOINT AND PSYCHROMETRIC MODE IN LEAF WATER POTENTIAL MEASUREMENTS

by

Gladys Durand-Campero

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology Ecology

(Plant Physiology)

Approved:

Major Professor

Committee Member

Committee Member

Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

1977

To my father,

In Memory

ACKNOWLEDGMENTS

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Gladys Durand-Campero

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ABSTRACT

A Comparison of Dewpoint and Psychrometric Mode in Leaf Water Potential Measurements

by

Gladys Durand-Campero, Master of Science

Utah State University, 1977

Major Professor: Herman H. Wiebe
Department: Biology

Leaf water potential of two maize plants (*Zea mays* L.) two chlorophytum plants (*Chlorophytum capense*, Kuntze), a schefflera (*Brassaia actinophylla*) and one aspen (*Populus tremuloides* Michx.), were measured under laboratory conditions with aluminum block *in situ* leaf hygrometers and with stainless steel single junction chamber hygrometer using excised entire leaves. Plants were subjected to a drying cycle. The hygrometers were controlled with a dewpoint microvoltmeter and all readouts were recorded on a chart recorder. A typical reading and control schedule included 20 second cooling before a first psychrometric reading allowing the output to return to zero, followed by 20 seconds cooling and switching to DEWPOINT function. Dewpoint was recorded for periods up to 300 seconds. Finally, the instrument was switched directly to READ function and a second psychrometric reading was recorded, again allowing the output to return to zero. The area under the psychrometric trace, measured during evaporation phase, was taken as a measurement of the amount of water condensed on the thermocouple.

It was found that the cooling coefficient (Π_v) of *in situ* leaf hygrometers had to be lowered, compared to Π_v values found in dry air, as plant water potentials decreased. This lowering was necessary to set the reading at dewpoint temperature without serious drifting. The areas under pre- and post- dewpoint psychrometric outputs were thus nearly equal and the dewpoint could be read for extended periods, confirming that equilibrium conditions were possible.

When water potential was measured in both the psychrometric and dewpoint mode with *in situ* leaf hygrometers, lower water potentials were found in the dewpoint mode than in the psychrometric mode and this difference tended to increase at lower water potentials. Conversely, in the Merrill units the water potentials determined on the psychrometric mode were consistently slightly lower than those based on the dewpoint readings. The greater agreement between psychrometric and dewpoint determinations obtained with Merrill units may well be explained by a manyfold higher leaf surface area exposed to the junction as compared to the limited leaf area sampled by the *in situ* leaf hygrometers. A greater area would contribute to a lesser total leaf resistance influencing the psychrometric determination.

The shape of hygrometer output traces in the psychrometric mode over standard solution generally had the typical, relatively flat shoulder, while over drier leaves it often had a more or less steady decline to zero. This difference was much more pronounced with *in situ* leaf hygrometers than with the chamber units which sampled larger leaf area.

The data suggest that the dewpoint mode, using proper precautions, measures water potential under equilibrium or isopiestic conditions, under which epidermal resistance is not a problem. Nonisopiestic conditions occur in the psychrometric mode. It appears that, immediately after cessation of the cooling current the evaporation of water from the wet junction elevates chamber vapor pressure above that of the mesophyll. This discrepancy would be zero over standard solutions and increases with increasing leaf resistance and with smaller leaf surface in the hygrometer.

(69 pages)

INTRODUCTION

Psychrometric techniques offer a convenient means for the determination of free energy status of water, the water potential, in plants, soils and other media. The development of miniature thermocouples by Spanner (1951) and Richards and Ogata (1958) has facilitated water potential determination. The Spanner psychrometer alters the initial equilibrium of temperature and water vapor concentration in the chamber for the short time during which an electrical current is passed in the direction that causes cooling of the measuring junction below the dew-point by the Peltier effect. This results in the condensation of a small amount of water on the junction. When the current is disconnected water starts to evaporate and the junction temperature is then depressed by the evaporative cooling; the amount of depression is a function of relative vapor pressure inside the chamber. The drier the atmosphere in the chamber the more rapid will be the evaporation rate and consequently the time available for psychrometric reading is shortened. Longer cooling periods are for this reason desirable to increase the accuracy and reliability of the reading on drier samples.

The validity of the psychrometric measurement has been questioned because leaf tissue sometimes apparently behaves differently than the wet filter paper generally used to calibrate the instrument. One possible cause of this different behavior is that leaf epidermal and stomatal resistance result in a smaller amount of water available for condensation on the thermocouple junction when leaf tissue is used.

It appears that accuracy depends largely on how closely conditions during calibration are reproduced during the measurement.

Neumann and Thurtell (1972) developed a technique for determining leaf water potential from the dewpoint measurement. This technique was later modified by Campbell, Campbell and Barlow (1973) who developed the theory and design necessary to construct a dewpoint meter based upon maintaining the thermocouple at dewpoint temperature. The method offers the advantage that at the dewpoint temperature no net water exchange occurs at the wet thermocouple junction and the measurement can be made at water vapor equilibrium between the chamber and the junction. Besides, the dewpoint measurement is relatively independent of such factors as the size and shape of the wet surface at the junction which affect the rate of water vapor exchange.

Since in the dewpoint method the water potential may be measured when no water vapor is moving between the sample and the hygrometer chamber, the measurement would be made at equilibrium conditions, equivalent to isopiestic conditions. Thus the equilibrium dewpoint method might be expected to more nearly measure the leaf water potential when leaf resistance is high.

The present study explores the possibility of the application of the dewpoint method to determine leaf water potential in plants subjected to drought and investigates the adjustments that are required for more accurate measurements.

The objectives of this study are:

1. To determine the influence on water potential measurement of

various factors such as cooling coefficient and duration in dewpoint mode, using various plant species at various water potentials.

2. To determine if the volume of water transmitted by the leaf area to the thermocouple junction varies under different conditions.

3. To ascertain if the dewpoint method actually measures water potential under isopiestic condition, i.e. zero water movement or equilibrium.

LITERATURE REVIEW

The theory, development and design criteria of the psychrometer and its application to research in water relations have received considerable attention during the last two decades.

The development of a psychrometer that measures the vapor pressure depression of a liquid sample began with Spanner (1951). The subsequent application of the psychrometer has revealed that many factors other than the water potential of the sample may influence the reading obtained with thermocouple psychrometer. For example, Barrs (1964) showed that the liberation of heat accompanying aerobic respiration by the tissue could influence psychrometric readings. Klute and Richards (1962) found psychrometer sensitivity depends on temperature.

Peck (1968) indicated two causes that may be involved in the increase of sensitivity: (1) the increase in the wet junction radius because of dew formation and (2) the increase in the apparent temperature depression of the measuring junction as a result of heat dissipation at the massive reference junction. Peck recommended a 1 cm^3 block of copper as a suitable massive junction to dissipate the heat produced during the cooling phase. Scotter (1972) criticized Peck's recommendations concluding they are misleading because massive reference junctions fail to account fully for conduction away from the junctions during cooling.

Rawlins (1964) suggested that if vapor diffusion between the sample and the chamber air is obstructed by a barrier such as the leaf epidermis, observations of water potential can be in error as a result of nonequilibrium between the sample and the chamber air if either sources or sinks for water vapor are present in the chamber.

Neumann and Thurtell (1972) developed an instrument that detects dewpoint depressions rather than wet bulb depressions. The dewpoint temperature measures the vapor pressure of water in the system rather than the ratio e/e° (relative humidity). The dewpoint temperature is, however, compared to the dry junction temperature, just as is the psychrometric temperature. Rawlins (1976) has indicated water potential determination based on dewpoint temperature as preferable to the wet bulb temperature because:

1. The relation of dewpoint temperature to water potential is less dependent upon the ambient temperature than is that of wet bulb temperature.

2. No net water condenses or evaporates from the wet junction during dewpoint measurement.

3. Psychrometric measurements are influenced by the wetting characteristics of the junction and the size and shape of the water droplet formed on the junction, whereas the dewpoint should be independent of these factors.

To measure dewpoint temperature Neumann and Thurtell (1972) used four terminal Peltier cooled thermocouple psychrometers. The dewpoint meter designed by Campbell et al. (1973) permits dewpoint measurement with the conventional two wire thermocouple. The circuitry of this dewpoint meter may be operated in such a way that cooling and sensing functions are time shared on the same thermocouple. Additionally, the electronic switching enables the dewpoint temperature to appear as a continuous reading on a panel meter or on a recorder chart.

Even though, in the theoretical design considerations, it is assumed that a wet thermocouple junction maintained precisely at dewpoint temperature will neither gain water through condensation nor lose water through evaporation, Campbell et al. (1973) admitted that under practical conditions it is not possible for a thermocouple junction to be absolutely independent of heat transfer mechanisms. This implies that errors in dewpoint measurement may arise by changes in sensitivity and cooling coefficient with temperature.

The cooling coefficient, (Π_v) represents the maximum junction temperature depression resulting from the passage of an optimum value of cooling current. This parameter is reported to be constant for a given thermocouple, environment and cooling current, and the dewpoint method requires the electronic gain of the duty cycle control circuitry be matched to the cooling coefficient (Π_v) of the thermocouple being used (Campbell et al. 1973). Usually the evaluation of Π_v is done with the thermocouple equilibrated in a dry chamber. Whether changes in cooling coefficient are necessary when the thermocouple is used in a humid chamber and if this influences leaf water potential measurement has not been reported.

The proper duration of cooling current seems to be more difficult to specify because it depends on the water potential of the sample. A cooling time considered adequate for low water potentials is much longer than required for high water potentials (Wiebe et al., 1971). If an adequate cooling period and cooling current are not used, reliability in measurement will be lost. The cooling period should not be made longer than is required because the error introduced by changing dry electromotive force (e.m.f.) is in proportion to the length of cooling period (Merrill and Rawlins, 1972).

Rawlins (1966) and Peck (1968) pointed out that the wet area of the junction varies in size and shape depending on the duration of cooling, the time elapsed following the cooling cycle, and geometry of the junction and lead wires.

Peck (1969) derived the equations to estimate the maximum time for which the thermojunction of a Spanner psychrometer may be cooled or allowed to evaporate with negligible effect of cuticular resistance. It is explained that in the case of very low cuticular resistances the restraints to the permissible cooling period will be set by the heat capacity of the sample or its change of water potential resulting from depletion of moisture content.

Since the resistance of leaf tissue to cede water to the thermocouple junction is likely to increase in plants subjected to variable drying periods, the use of longer cooling periods has been adopted to obtain leaf psychrometric measurements of plants under water stress.

MATERIALS AND METHODS

Volume and Rate of Water Condensation Measurement

A microscope with a calibrated ocular micrometer was used to measure junction bead and water drop diameter of two leaf hygrometers: a model L51 (Wescor Inc., Logan, Utah) and one produced by EMCO (EMCO, Angola, Indiana).

The leaf hygrometer, surrounded by wet filter paper, was fixed in a stoppered clear bottom glass vial and mounted in inverted position on the microscope stage. The junction bead and condensed water drop could be observed and measured through the bottom of the vial using incident illumination. Light was used only for the short time in which the measurement was made to avoid heating of the junction and cause water evaporation, thus minimizing the error in the determination. When the microvoltmeter was in COOL function it was possible to observe and measure the diameter of water droplet condensing on the junction. The drop enlarged to diameter as much as 4x that of the junction bead before it fell off from the bead. This might require from 20 to over 40 minutes. The results were used to calculate rate of water condensation on the thermocouple assuming spherical drop shape.

Leaf Water Potential Measurements

Measurements were made on two maize plants (*Zea mays* L.), two chlorophytum plants (*Chlorophytum capense*, Kuntze), a schefflera (*Brassaia actinophylla*) and one aspen (*Populus tremuloides* Michx.).

Plants were grown on soil in pots in a glass house. Leaf water potential was monitored under laboratory conditions with aluminum block leaf hygrometers L51, throughout a drying cycle until the entire plant showed severe wilting. Plants were given 16 hours daily illumination of up to 185 μeinsteins/m^2/sec using both fluorescent and incandescent lights. Illumination was interrupted during the period in which the measurements were made.

Leaves were gently washed with distilled water and a sponge and permitted to dry an hour before the hygrometers were attached. The hygrometer mounting procedure was that used by Wiebe and Prosser (1977) except in our case the hygrometers were attached on different leaves of the plant instead of on one leaf. At least four *in situ* leaf hygrometers were mounted on upper surface near the edge of leaves and left overnight before readings were taken.

Wiebe and Prosser (1977, p. 256) described the mounting procedure as follows:

> Prior to attachment, a rubber washer (cut from 0.2 mm sheet rubber-dental dam) was cemented inside the leaf slit of the aluminum block to provide a base, or back stop, to press the leaf firmly but gently against the hygrometer unit. The aluminum block hygrometer housing, mounted on wooden dowels on a Styrofoam block base, were then assembled along both sides of the leaf with care to avoid leaf twisting or injury. Then the hygrometers cylinders themselves, each with a Parafilm gasket lightly coated on both surfaces with petrolatum, were inserted in the aluminum blocks, seated firmly against the leaf (with the rubber washer on the other side of the leaf), and secured with the setscrew.

By this procedure I have been able to attach and get satisfactory readings from most of the hygrometers on most species used throughout the drying cycle. An exception was Brassaia whose leaves are normally somewhat succulent but which became thinner and often slipped within the unit on drying. Consequently, it was necessary to repeat the mounting procedure on other leaves as the plant dried.

Simultaneous determinations were made for comparative purposes with stainless steel single junction Merrill psychrometer placed in the center of a stainless steel chamber (J. R. D. Merrill Specialty Equipment, Logan, Utah) using excised entire leaves. In case of maize a strip was cut from the edge of the leaf minimizing in this way the cut surface. The leaf or portion of the leaf was wrapped around the hygrometer cylinder and allowed to uncoil against the side walls of the chamber. The stainless steel chamber assembled to the hygrometer unit was immediately sealed and immersed in a water bath at 25C. Readings were begun after two hour equilibration.

The configuration in which the psychrometer is concentric to the sample surface favors thermal equilibrium. Besides, it provides a maximum surface of the sample exposed to the thermocouple hygrometer and facilitates a rapid vapor pressure equilibrium.

The hygrometers were controlled and read with a Wescor HR33 dew-point microvoltmeter (Wescor Inc.) and all readouts were recorded on a chart recorder.

Cooling coefficients were determined for all hygrometers in a dry atmosphere according to Wescor Instruction Manual for dewpoint microvoltmeter. A typical reading and control schedule was:

1. Zero instrument on READ.
2. COOL for 20 seconds.
3. READ allowing output to return to zero. The relatively level reading attained in about 5 seconds, was recorded as the usual psychrometric reading, here termed P_1.

4. COOL for 20 seconds, then to DEW POINT function. The dewpoint reading was recorded at 30, 120 and 300 seconds. These observations were termed DP_1, DP_2 and DP_3, respectively.

5. After 120 or 300 seconds, the instrument was switched directly to the READ function, and a second psychrometric reading, recorded as P_2, was obtained. Again the output was allowed to return to zero.

In addition to the psychrometric and dewpoint readings, the area under each psychrometric recorder trace was measured with a compensating polar planimeter. These areas were termed the P_1 area (P_1A) and the P_2 area (P_2A), respectively. Since the main dewpoint method requirement is to have a steady dewpoint output I proceeded in this way: if drifting during dewpoint reading indicated excess cooling of the measuring junction this resulted in excess water condensation and was reflected in the area under psychrometric P_2 (P_2A) for being greater than P_1 area (P_1A). To correct this drifting, before starting the next reading schedule, I increased the value of cooling coefficient by one, two or more units depending on how great the drifting was. If drifting indicated warming of the junction, water evaporated from it and the P_2 area was smaller than P_1 area. To correct this drifting, before starting the next reading schedule, I lowered the cooling coefficient by one, two or more units. Changes in cooling coefficient values made it possible to obtain dewpoint readings without drifting through 30, 120, 300 seconds or even longer. The cooling coefficient for which dewpoint was steady could generally be used in successive readings during one day and sometimes during the next two or three days, but when the plant water stress was increasingly high drifting in dewpoint often indicated evaporation for which more frequent changes in cooling coefficient had to be made.

Measurements obtained with hygrometers were compared with periodic determination of leaf water potential made in the pressure bomb.

Calibration of Leaf Hygrometers

Aluminum block leaf hygrometers were calibrated over sodium chloride solutions at -9.2 bars, -22.8 bars and -46.4 bars at 25C.

The calibration procedure was designed to simulate leaves. Tightly folded aluminum foil envelopes were prepared, each one having a 6 mm diameter hole on one surface and enclosing three 16 mm diameter filter paper discs. The paper was wetted with standard solution and the envelope so prepared was mounted in the slit of the aluminum block hygrometer housing. The mounting procedure was the same as that used for leaves. Readings were obtained beginning after two hour temperature and water vapor equilibration.

Calibration of the Merril hygrometers was made following the mounting procedure already described for leaves but substituting a strip of filter paper wetted with the standard solution.

Cooling coefficient determined in dry chamber as per Wescor Instruction Manual for dewpoint microvoltmeter, did not work well in measurements with standard solutions. The procedure used to determine Π_V was the same followed with plants, except once dewpoint output was steady for a given cooling coefficient it could be used in successive readings on all the standard solutions and no drifting in dewpoint was observed. The reading schedule followed in calibration was the same described for plants.

Water Condensation Measurement at Low Water Potentials

Wescor C52 sample chamber hygrometers were calibrated using standard solutions: NaCl at -22.8 and -46.4 bars and LiCl at -100, -300 and -600 bars. The method used was that developed by Wilson and Harris (1968) and by Campbell and Wilson (1972) in which the Spanner psychrometer is used basically like a Richards and Ogata wet loop psychrometer. A large drop of water is condensed by Peltier cooling; following this, the sample is moved into the thermocouple chamber and its water potential determined.

One of the two sample positions of the chamber was lined with filter paper saturated with distilled water. Water was condensed on the thermocouple for variable period of time up to 20 minutes; then the second position sample holder slide was charged with filter paper saturated with one of the different standard solutions and slid softly but quickly to place in the thermocouple chamber. Extreme care was taken to minimize exposure of the sample to the atmosphere and thus water loss which might change the actual water potential. The water potential was taken as the maximum deflection achieved within a minute or less.

The same procedure was followed with standard solutions NaCl at -22.8 and -46.4 bars. These were also cooled for variable periods over the respective solutions to compare the amount of water condensed over various water potentials when evaporation occurred at standard potentials.

All readouts were recorded on a chart recorder and the area under each psychrometric trace was measured with a polar planimeter, thus both water potential and water volume measurement were made.

RESULTS

Volume and Rate of Water Condensation

Volume and rate of water condensation were calculated from microscopic measurement during the first 21 minutes in the COOL function assuming the drop of water was a perfect sphere. A condensation rate of 1.24×10^{-2} μg/sec for the Wescor leaf hygrometer and a rate of 1.7×10^{-2} μg/sec for the EMCO psychrometer were found.

Water condensation rate obtained for EMCO hygrometer was assumed to be the same as that of the stainless steel screen Merrill hygrometer, taking into consideration that both hygrometers have nearly the same dimensions. Unfortunately, the measurement could not be made on the Merrill hygrometer without removing the stainless steel screen. The measurement involves some error due to evaporation caused by the heating effect of the light used to illuminate the thermocouple at the moment in which the measurements were made. This evaporation was minimized by keeping light off at other times.

Calibration of Hygrometers

Since considerable variability in hygrometer characteristics exist, they were individually calibrated. Results are given in Tables 1 and 2. Linear regression equations were calculated and plotted to predict water potential based on P_1 and DP_2 readings for all Wescor leaf hygrometers and for two Merrill hygrometers. Although the regression analysis was made with only two degrees of freedom, determination

Table 1. Calibration data for Wescor and Merrill hygrometers

Hygrometer No.[a]		L6	L8	L9	L10	L11	L12	L13	L14	L15	M1	M2
Cooling Coefficient (μvolts)		74	76	70	72	67	72	70	73	69	65	78
P_2/P_1 at	- 9.2 bars	0.94	0.99	0.96	0.97	0.96	0.94	0.96	0.93	0.88	0.95	1.00
	-22.8 bars	0.97	1.00	1.00	1.01	0.94	0.98	0.96	0.97	0.98	0.98	1.00
	-46.4 bars	0.99	1.00	0.96	0.96	0.96	1.00	0.90	1.09	0.86	1.00	1.05
DP_2/DP_1 at	- 9.2 bars	1.00	1.00	1.01	1.00	0.99	1.06	1.00	0.99	1.00	1.00	1.00
	-22.8 bars	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00
	-46.4 bars	1.00	1.00	0.99	1.00	0.99	1.00	0.99	1.00	1.01	1.00	1.00
P_2 area/P_1 area at	- 9.2 bars	1.01	1.03	1.03	0.96	1.03	0.97	0.96	1.04	0.96	0.99	0.99
	-22.8 bars	1.01	1.02	1.12	1.04	1.03	0.98	0.94	1.17	0.94	0.94	0.98
	-46.4 bars	0.80	0.81	0.72	0.81	0.89	0.87	0.82	0.98	0.71	0.60	0.81

[a] L or M denote Wescor or Merrill hygrometer, respectively

Table 2. Regression coefficients obtained to predict water potential (bars) based in psychrometric (μvolts) and dewpoint readings (μvolts) and water condensation data for Wescor and Merrill hygrometers.

Hygrometer No.[a]	L6	L8	L9	L10	L11	L12	L13	L14	L15	M1	M2
Psy. intercept bo (bars)	3.38	2.37	3.98	3.83	3.07	2.06	3.18	4.96	4.64	2.32	0.18
Slope b1 (bars/μvolts)	-2.73	-2.53	-2.64	-2.73	-2.87	-2.63	-2.87	-2.91	-2.95	-2.24	-2.31
Determination Coeff. r^2	0.98	1.00	1.00	0.98	1.00	1.00	1.00	0.98	0.96	1.00	1.00
DP_2 Intercept bo (bars)	0.58	1.51	0.40	0.38	-0.48	0.85	-0.12	1.12	2.43	2.64	1.66
Slope b1 (bars/μvolts)	-1.24	-1.32	-1.20	-1.25	-1.19	-1.31	-1.20	-1.21	-1.34	-1.28	-1.13
Determination Coeff. r^2	.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Area (in^2) at Ψ = 0 at 20 sec cooling	1.48	1.67	1.50	1.49	1.40	1.53	1.37	1.67	1.23	1.41	1.34
μg H_2O cond. per 20 sec = in^2 x factor	.169	.150	.167	.168	.179	.163	.182	.150	.203	.242	.254

[a] L or M denote Wescor or Merrill hygrometer, respectively

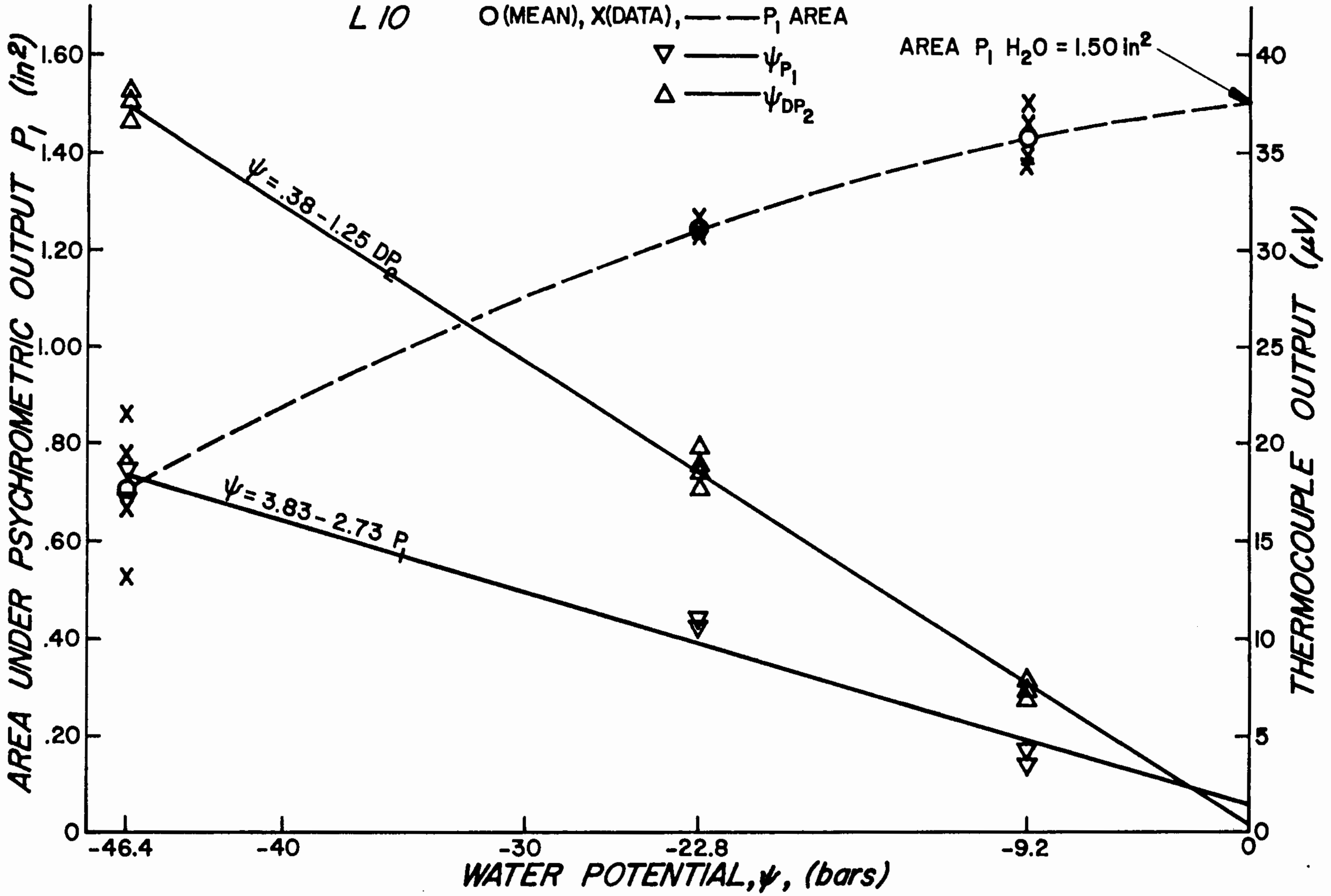


Figure 1. Calibration curve for a Wescor leaf hygrometer

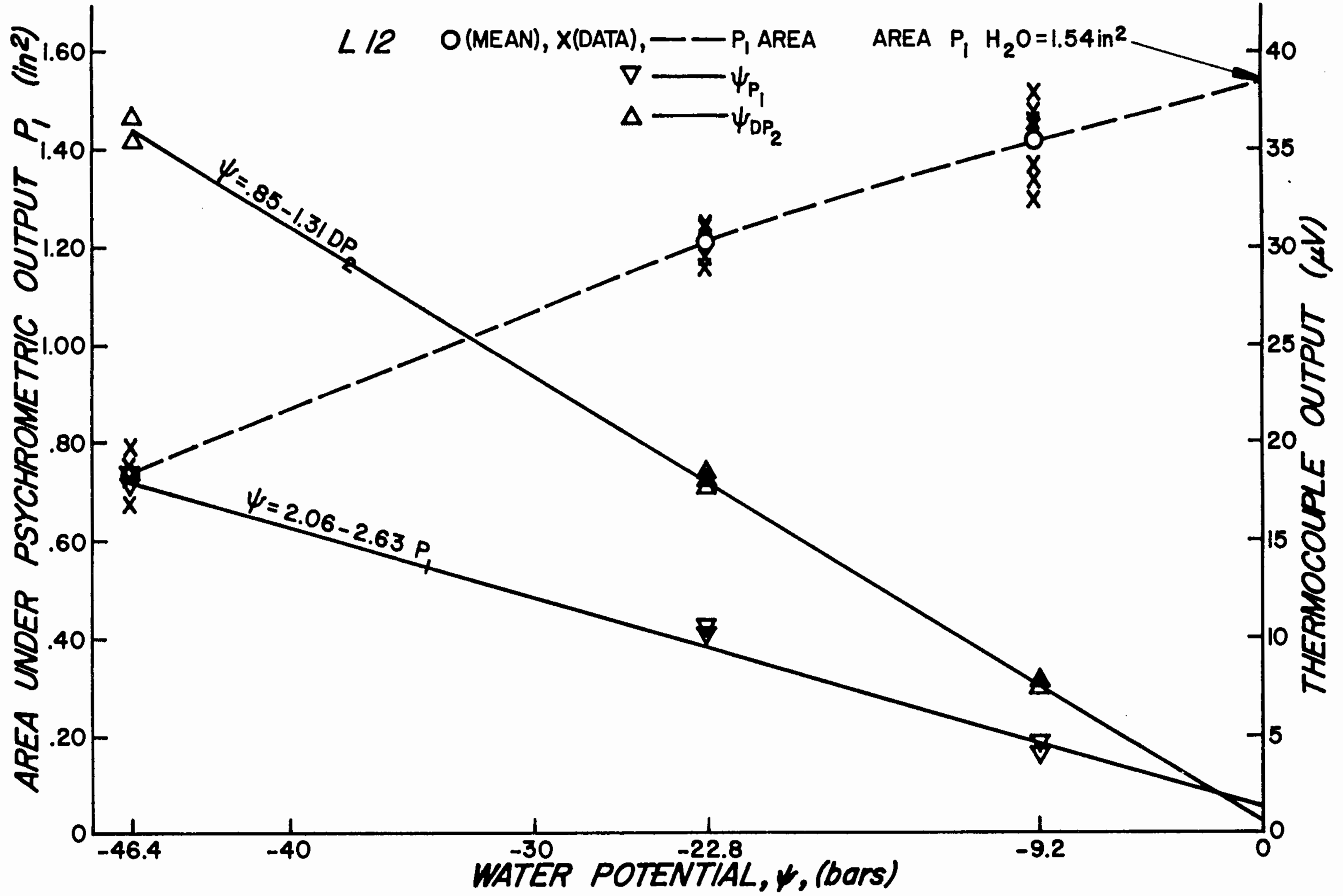


Figure 2. Calibration curve for a Wescor leaf hygrometer

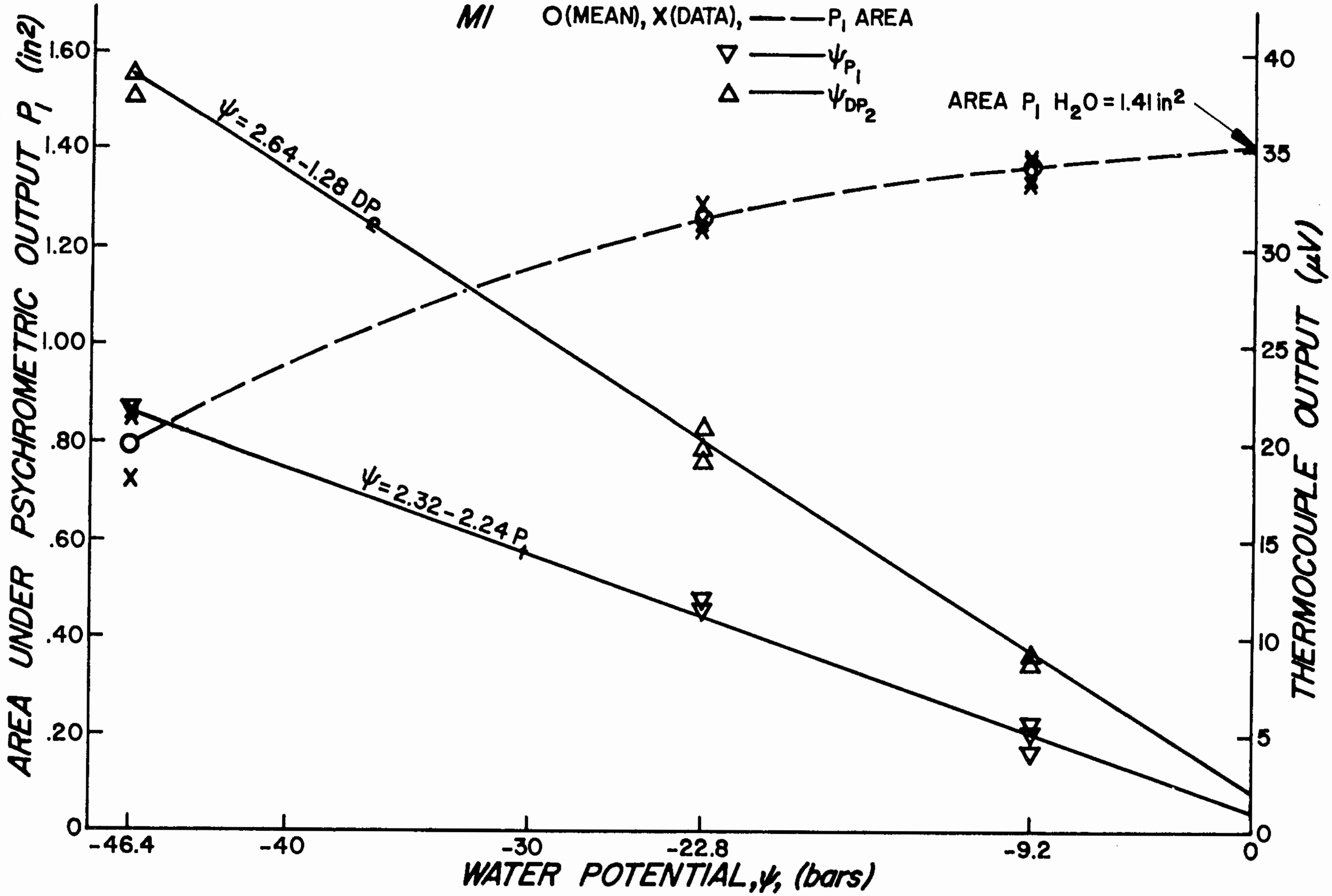


Figure 3. Calibration curve for a stainless steel Merrill hygrometer

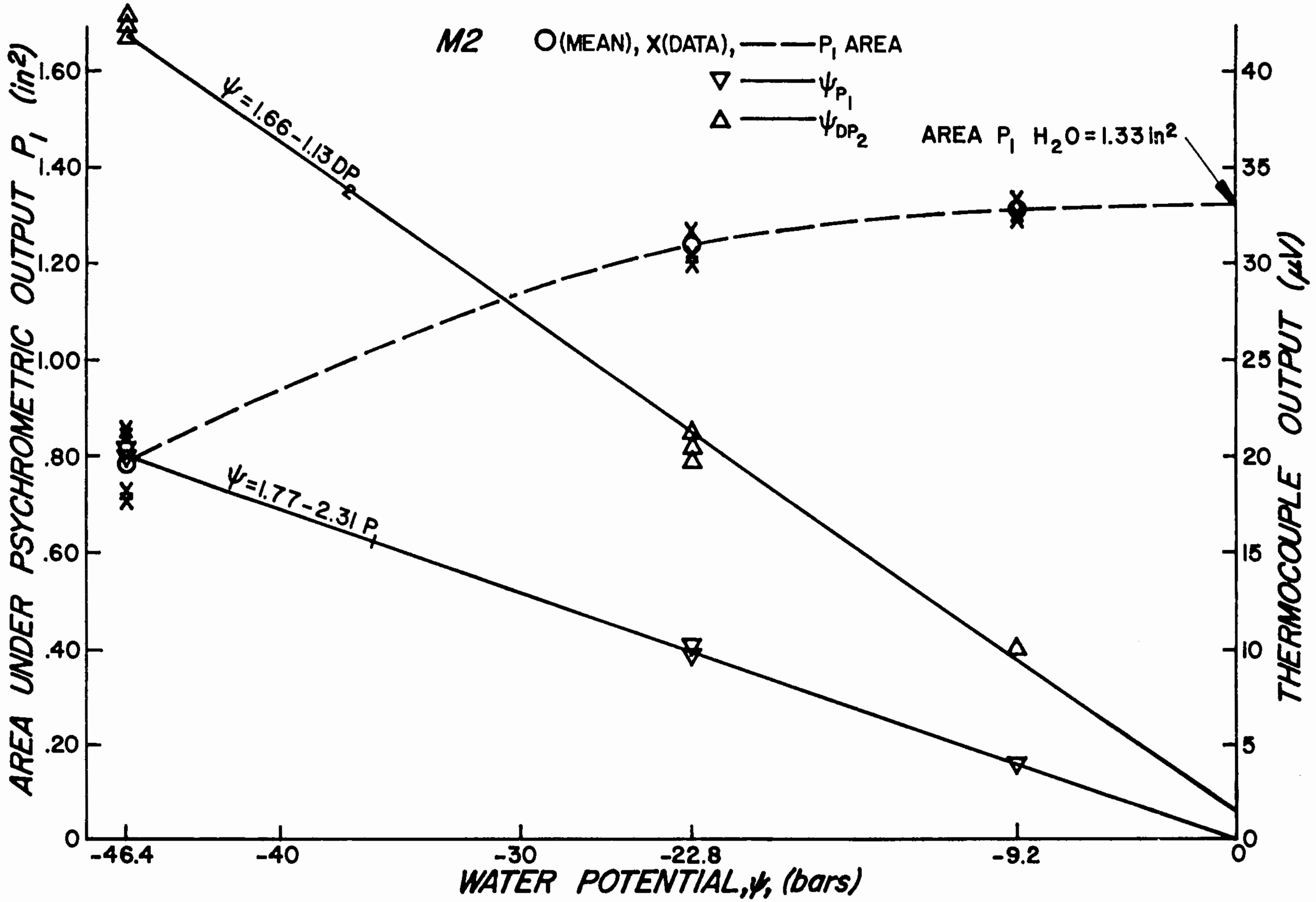


Figure 4. Calibration curve for a stainless steel Merrill hygrometer

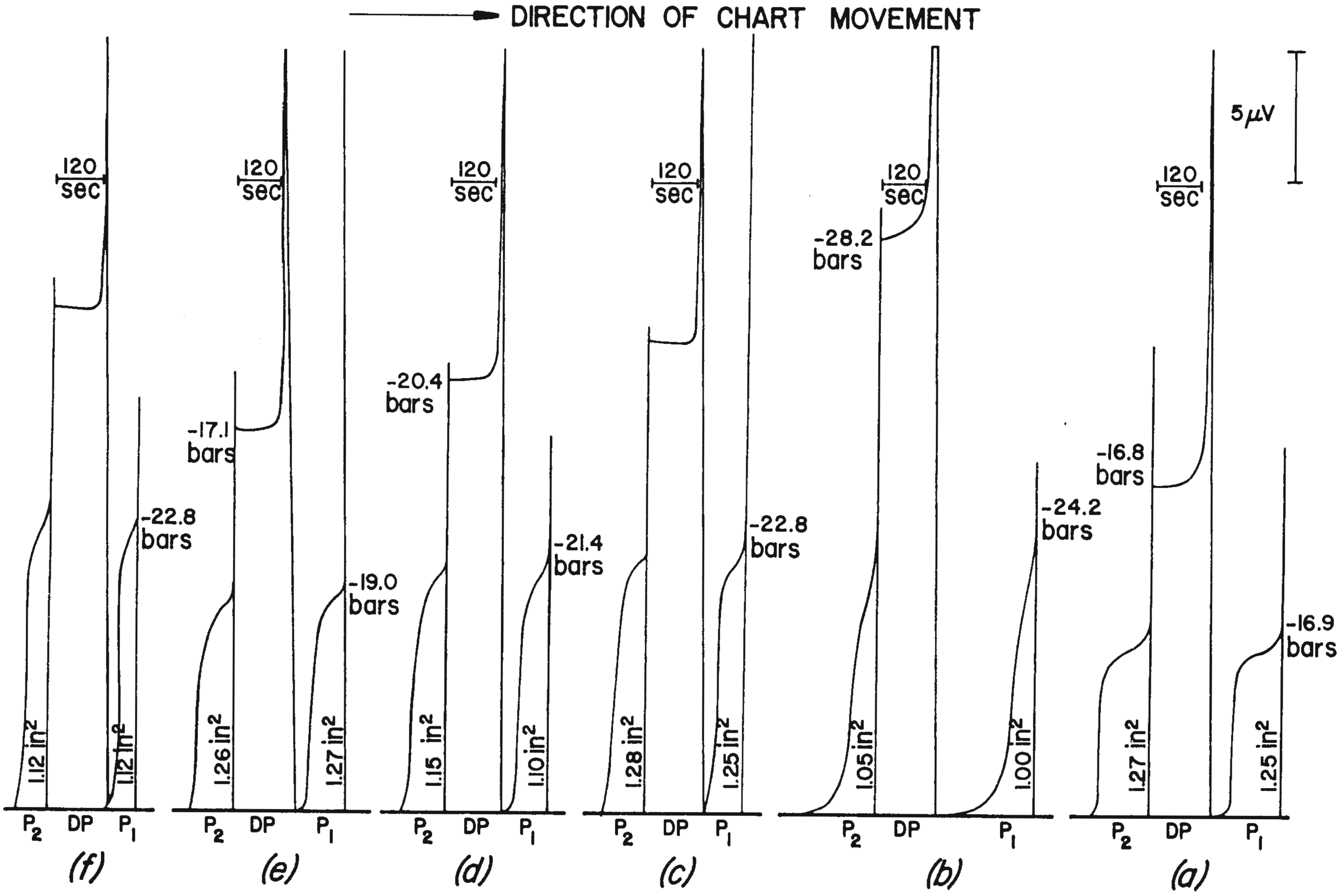
coefficient r^2 values ranging from 0.92 to 1.00 were highly significant.

Since all calibration curves were similar, only two typical curves for Wescor leaf hygrometers are shown in Figures 1 and 2. Calibration curves for two Merrill hygrometers are given in Figures 3 and 4. Plots of area under psychrometric reading P_1 versus water potential (Ψ) in Figures 1, 2, 3, and 4 indicate that less water is condensed on the thermocouple as water potential decreases. In the graphs each point represents the average P_1 area of four points obtained with each of the standard solutions. Visual inspection was used to join the average points and to extrapolate the line to the interception on the Y axis assuming that maximum condensation would occur over distilled water. This point cannot be measured since over distilled water evaporation time approaches infinity.

Comparison of Recorder Traces Obtained on Standard Solution and on Plants

In Figure 5 are reproduced selected recorder traces obtained with L8 and Merrill hygrometer (M1) on NaCl solution at -22.8 bars and on two different plant species. The recorder traces under psychrometric readings obtained with L8 on NaCl solution at -22.8 bars and on a Brassaia leaf had similar shapes with pronounced nearly steady P_1 readings. In both cases the evaporation rate was slower than when the thermocouple was on a Chlorophytum leaf even though its water potential was only 1.4 bars lower than the water potential of the standard solution. The output curve of the Chlorophytum leaf was far steeper

Figure 5. Selected recorder traces of hygrometer obtained on standard solution and on plants. P_1 and P_2 represent first and second psychrometric readings and DP dewpoint reading. Graphs (a), (b), and (c) were obtained with a Wescor leaf hygrometer (L8) on a Brassaia leaf, a Chlorophytum leaf and on NaCl solution at -22.8 bars, respectively. Graphs (d), (e) and (f) show the same sequence of traces obtained with a Merrill hygrometer (M1).


DIRECTION OF CHART MOVEMENT
5 μV
120 sec
1.12 in²
1.12 in²
-22.8 bars
P₂ DP P₁
(f)
120 sec
-17.1 bars
1.26 in²
1.27 in²
-19.0 bars
P₂ DP P₁
(e)
120 sec
-20.4 bars
1.15 in²
1.10 in²
-21.4 bars
P₂ DP P₁
(d)
120 sec
1.28 in²
1.25 in²
-22.8 bars
P₂ DP P₁
(c)
120 sec
-28.2 bars
1.05 in²
1.00 in²
-24.2 bars
P₂ DP P₁
(b)
120 sec
-16.8 bars
1.27 in²
1.25 in²
-16.9 bars
P₂ DP P₁
(a)

and contained no relatively stable or level portion as with the unit over standard solution.

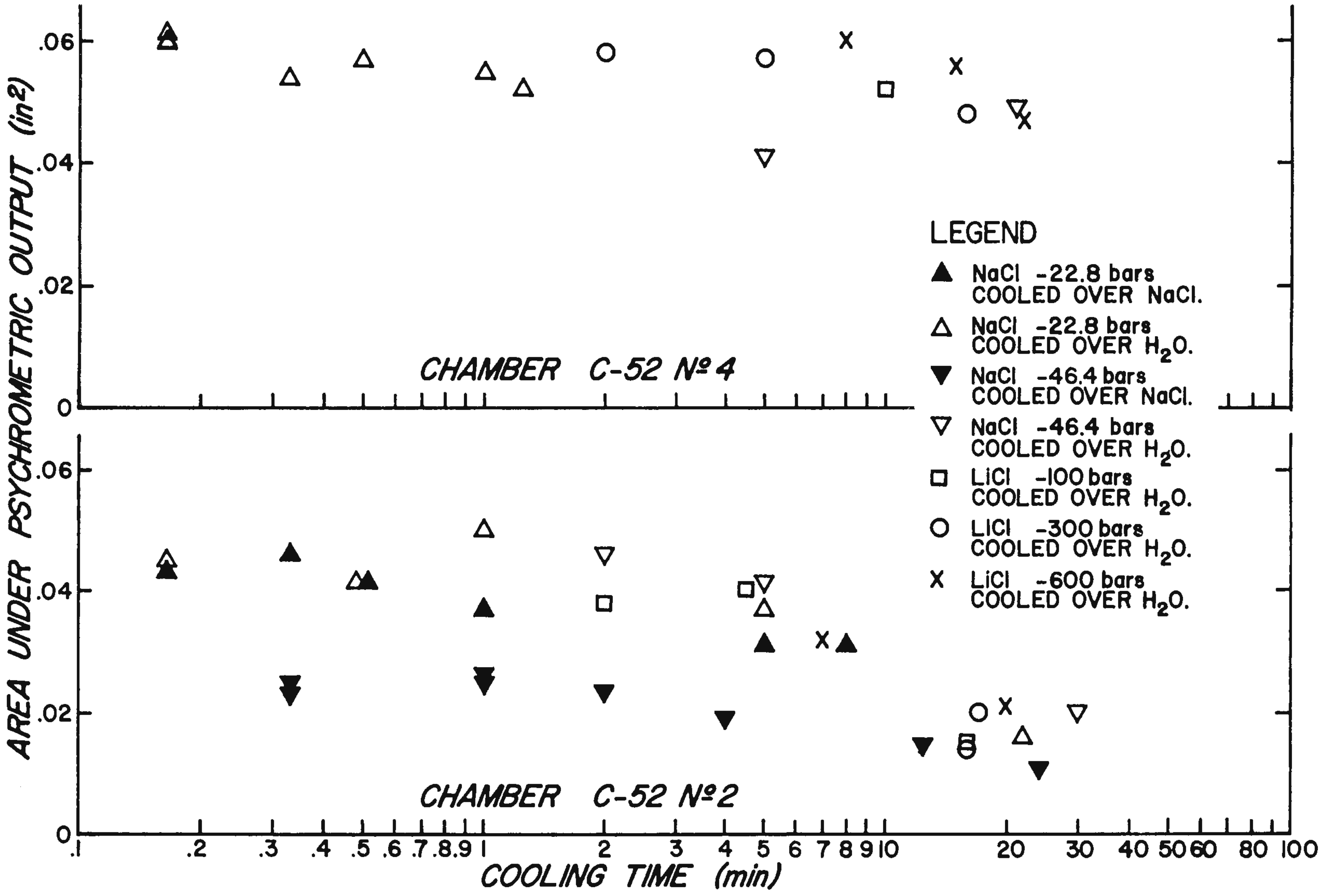
Comparing the water potentials measured in psychrometric readout in both Chlorophytum leaf and NaCl solution there was only 1.4 bars difference but the water potential measured in the dewpoint mode was 4 bars lower than the psychrometric water potential. The volume of water condensed on the junction, as indicated by P_1 area, was also much lower than the volume condensed on standard solution even though water potential difference was small. The cooling coefficient used in the three different determinations was the same, $\Pi_v = 75$, but 5 units lower than the cooling coefficient determined in dry chamber. Since P_2 area did not differ of P_1 area it is evident that during dewpoint mode neither evaporation nor water condensation occurred at the junction.

The recorder traces selected for Merrill hygrometer show that slightly different profiles were obtained with the thermocouple over standard solution and on a Brassaia leaf at nearly the same water potentials. The measurements made on plants resulted in higher water potentials determined in the dewpoint mode than in the psychrometric. The P_1 area recorded on the leaf was nearer to that obtained with NaCl solution at -22.8 bars, i.e., actual area was closer to the expected area.

Water Condensation Measurement at Low Water Potentials

Results are given in Figure 6. Area under psychrometric output recorded during the evaporation phase is considered to be a function of the amount of water condensed on the thermocouple. This assumption is

Figure 6. Relationship between duration of cooling, over water or solution, to the area under the psychrometric trace over solution ranging to Ψ = -600 bars.


.06
.04
.02
0
CHAMBER C-52 Nº 4
CHAMBER C-52 Nº 2
AREA UNDER PSYCHROMETRIC OUTPUT (in²)
COOLING TIME (min)
.1 .2 .3 .4 .5 .6 .7 .8 .9 1 2 3 4 5 6 7 8 9 10 20 30 40 50 60 80 100
LEGEND
▲ NaCl -22.8 bars COOLED OVER NaCl.
△ NaCl -22.8 bars COOLED OVER H2O.
▼ NaCl -46.4 bars COOLED OVER NaCl.
▽ NaCl -46.4 bars COOLED OVER H2O.
□ LiCl -100 bars COOLED OVER H2O.
○ LiCl -300 bars COOLED OVER H2O.
X LiCl -600 bars COOLED OVER H2O.

supported by the fact that the area under the curve was nearly constant when evaporation occurred at a low rate for a long time over -22.8 bar solution or at a rapid rate and short time over -600 bar solution. Expressing the amount of water condensed as a function of the water potential of the solution and cooling time, a multiple linear regression equation was developed to fit the data. The general equation is:

$$Y = bo + b_1 X_1 + b_2 X_2$$

where Y = expected area under psychrometric output recorded during evaporation phase (in^2);

X_1 = water potential (bars) of evaporating solution

X_2 = cooling time (minutes) over water or over solution as indicated.

For chamber C52 No. 2, the data obtained by cooling over water fit the equation

$$Y = 4.4 \times 10^{-2} + 9.8 \times 10^{-6} X_1 - 1.1 \times 10^{-3} X_2 \qquad [1]$$

Determination coefficient $r^2 = 0.79$

For chamber C52 No. 2, the data obtained by cooling over the respective standard solution fit the equation

$$Y = 5.4 \times 10^{-3} + 6.3 \times 10^{-4} X_1 - 7.5 \times 10^{-4} X_2 \qquad [2]$$

$r^2 = 0.81$

For chamber C52 No. 4 the data obtained by cooling over water fit the equation:

$$Y = 5.6 \times 10^{-2} - 1.1 \times 10^{-5} X_1 - 5.7 \times 10^{-4} X_2 \qquad [3]$$

$$r^2 = 0.44$$

Comparison of the equations with higher determination coefficients r^2, [1] and [2], indicates that the amount of water condensed on the junction decreased somewhat with the cooling time and that the decrease was greater when the cooling was done over H_2O than over standard solutions. The effect of water potential of evaporating solution on the amount of water condensed was negligible when the cooling was done over water but a significant decrease was obtained when cooling was done over solution as water potential lowered. In Figure 6 it is clear that for any given cooling time the amount of water condensed on the junction was greater when the cooling was done over H_2O rather than over standard solution.

Leaf Water Potential Measurements

Results obtained from individual hygrometers throughout the drying cycle for the various plants studied are shown in Figure 7 through Figure 16. Each point in the graphs represents the average of at least four determinations. In each graph are illustrated the response of leaf water potential to increased water stress. Leaf water potential was monitored in both psychrometric and dewpoint mode and both are plotted for comparative purposes. Also, the volume of water condensed on the junction measured during evaporation phase is plotted; and finally the ratio P_1 area actually measured during evaporation phase to P_1 area expected at the same water potential (P_1Aa/P_1Ae) is illustrated.

Zea mays No. 1

Figure 7 shows the results obtained with two Wescor leaf hygrometers (L6, L8). Leaf water potential measurements do not agree the first two days of the drying period but they do the last three days. Lower water potentials were obtained from dew point mode than from the psychrometric mode. The difference increased as the plant water stress increased, becoming finally 4.4 bars for L6 and 2.2 bars for L8. Decreases of about 25 percent and 30 percent compared to initial volume of water condensed on the thermocouple were obtained in L6 and L8, respectively. The ratio P_1Aa/P_1Ae decreased in both hygrometers throughout the drying cycle.

Zea mays No. 2

In Figures 8 and 9, fair agreement is shown in leaf water potentials measured with Wescor hygrometers the 1st, 2nd, and 5th day of the drying period. Between the Merrill hygrometers agreement in water potential determinations was obtained only during the first three days. Leaf water potential measurements taken with both type of hygrometers were in general agreement. Water potentials as determined by the dewpoint mode were generally lower than potentials simultaneously determined by the psychrometric mode; the discrepancy was generally about 1 bar throughout the drying cycle in Wescor hygrometers, but no appreciable difference was obtained in Merrill hygrometers. Water potential measured in the pressure bomb decreased from -3 to -13 bars. Measurement of water volume condensed on the junction obtained with Wescor hygrometer indicated a decrease range from 14 percent to 30 percent. In Merrill hygrometer that decrease ranged from 12 percent to

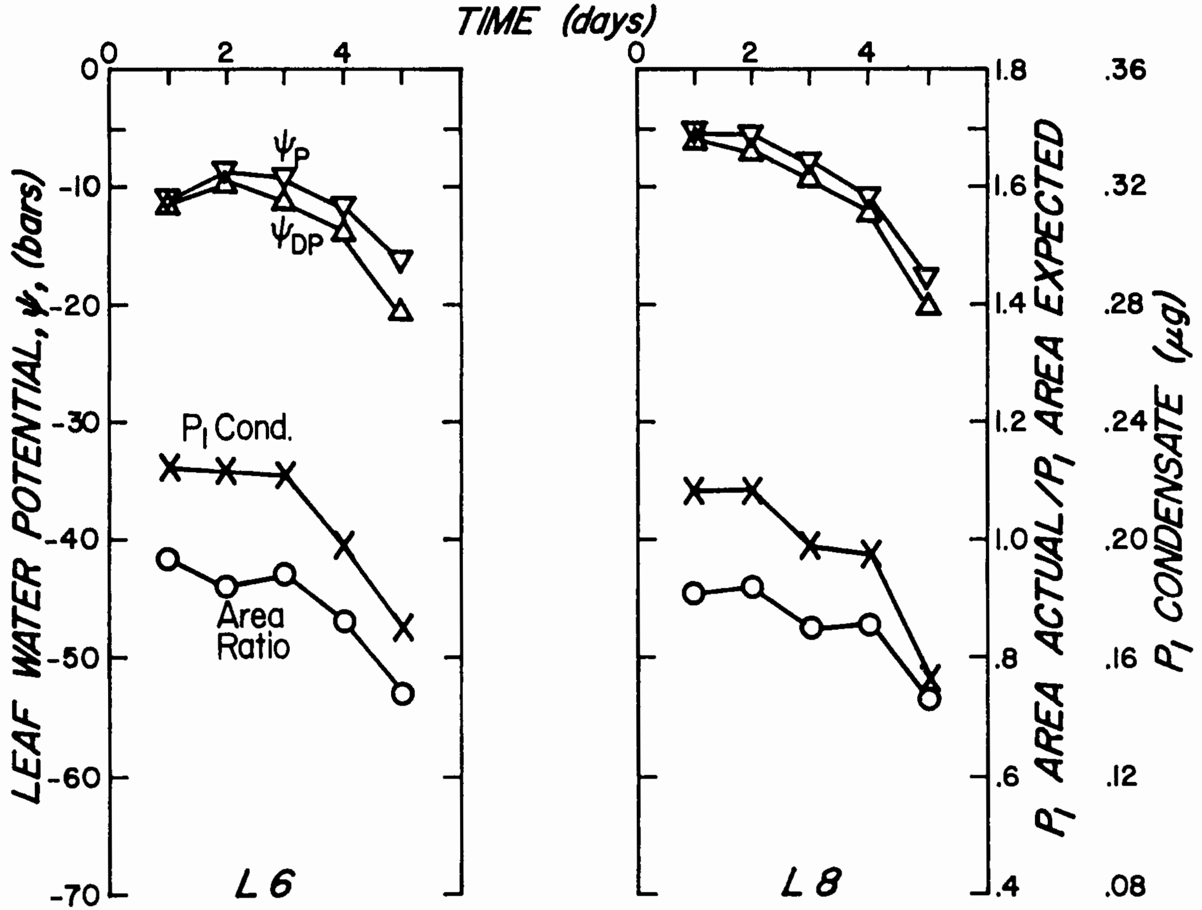


Figure 7. Relationship between time in days and leaf water potential, measured in the psychrometric (ψP) and dewpoint mode (ψDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Zea mays No. 1. L denotes Wescor leaf hygrometer.

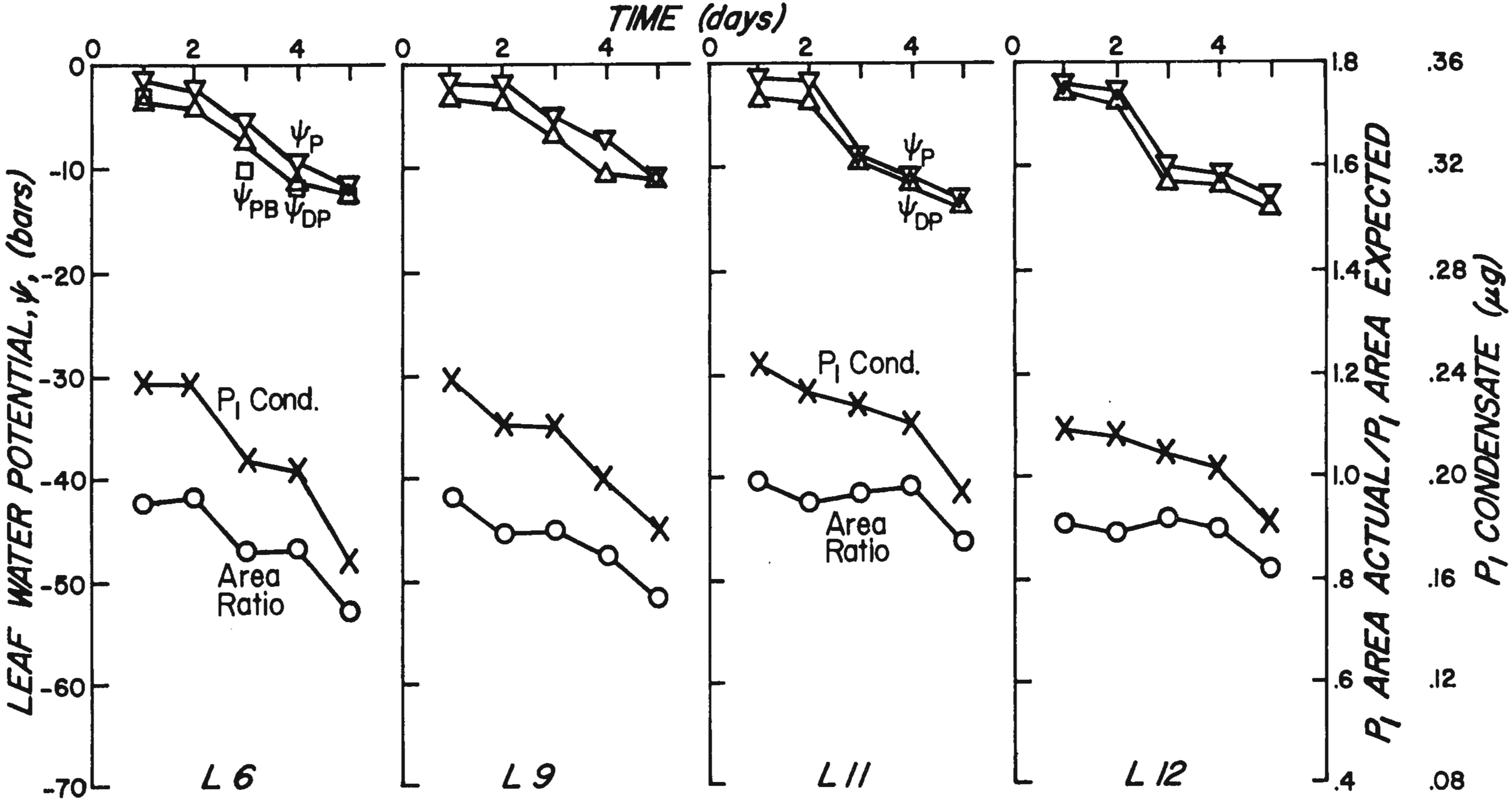


Figure 8. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Zea mays No. 2. L denotes Wescor leaf hygrometer. Ψ_{PB} denotes water potential measured in pressure bomb.

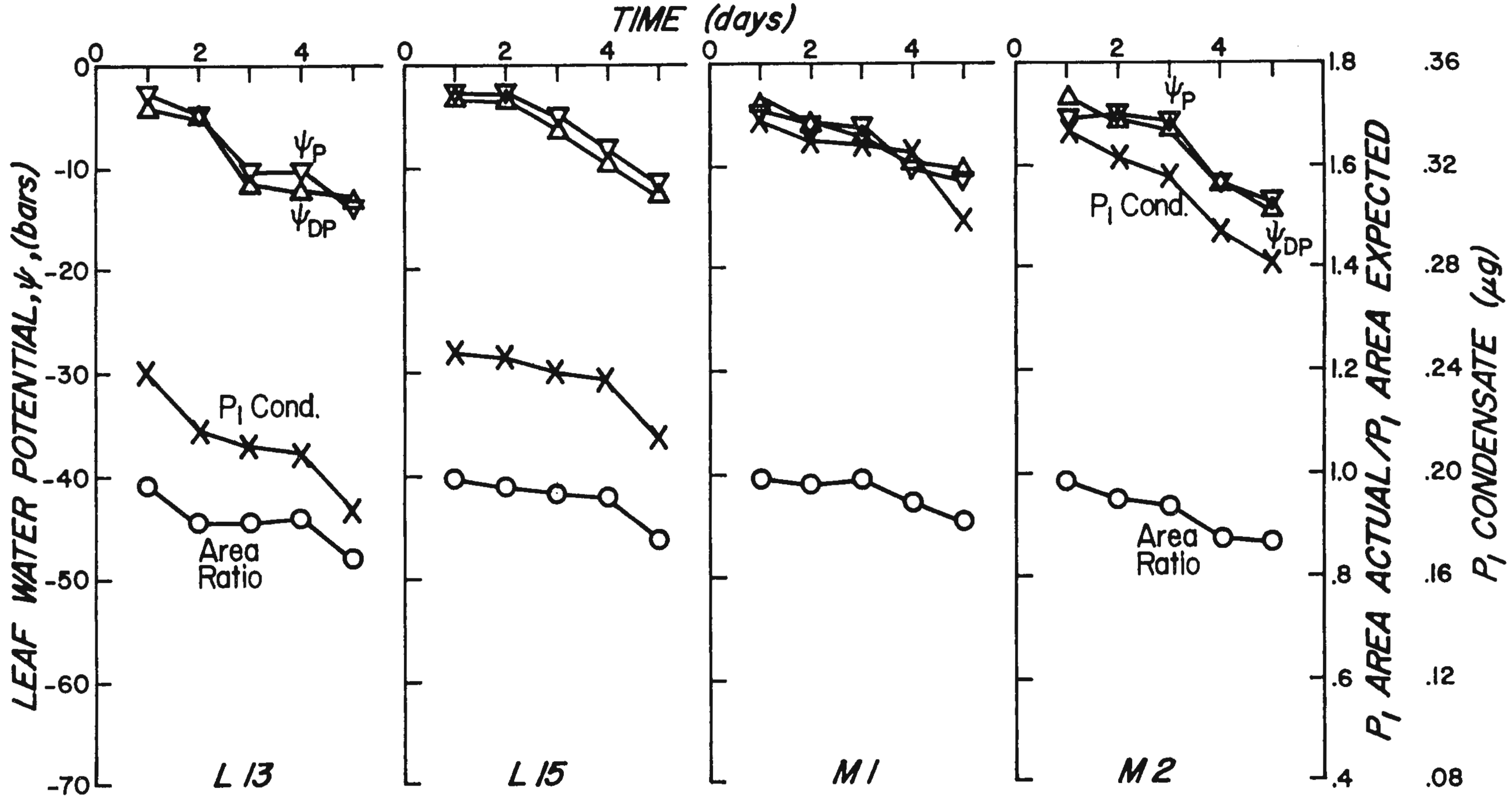


Figure 9. Relationship between time in days and leaf water potential (ψ), measured in the psychrometric (ψP) and dewpoint mode (ψDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Zea mays No. 2. L or M denotes Wescor or Merrill hygrometer, respectively.

to 16 percent. The ratio P_1Aa/P_1Ae obtained with Wescor hygrometers was initially close to 1 but as the plant water stress increased the ratio decreased from 9 percent to 21 percent. With the Merrill hygrometer the decrease was less marked.

The entire plant showed wilting signs at a soil water potential of -13 bars. No additional readings could be obtained after the fifth day of the drying period but the plant recovered after rewatering.

Chlorophytum capense No. 1

In comparing the leaf water potential as measured by Wescor leaf hygrometers, (Figures 10 and 11), it is evident that L8 and L9 were in agreement throughout the drying period while the determinations by L10 were consistently higher but still in fair agreement with the measurements obtained in Merrill hygrometers. Generally, lower water potentials were measured in dewpoint mode than in psychrometric mode with Wescor hygrometers; the opposite tendency was evident in Merrill hygrometers. In both types of hygrometers such differences were not greater than 2.5 bars. Decreases in volume of water condensed on the junction compared to the initial volume ranged from 38 percent in Wescor units to 14 percent in Merrill hygrometers.

Among Wescor hygrometers the initial ratios $P_1Aa:P_1Ae$ ranged from 0.96 to 0.81; toward the end of drying period a decrease of about 20 percent was found. In Merrill hygrometer the ratio changed from 0.96 to 0.88 during the same period. The lowest water potentials measured in the last day were about -22 bars; however, the plant recovered after rewatering.

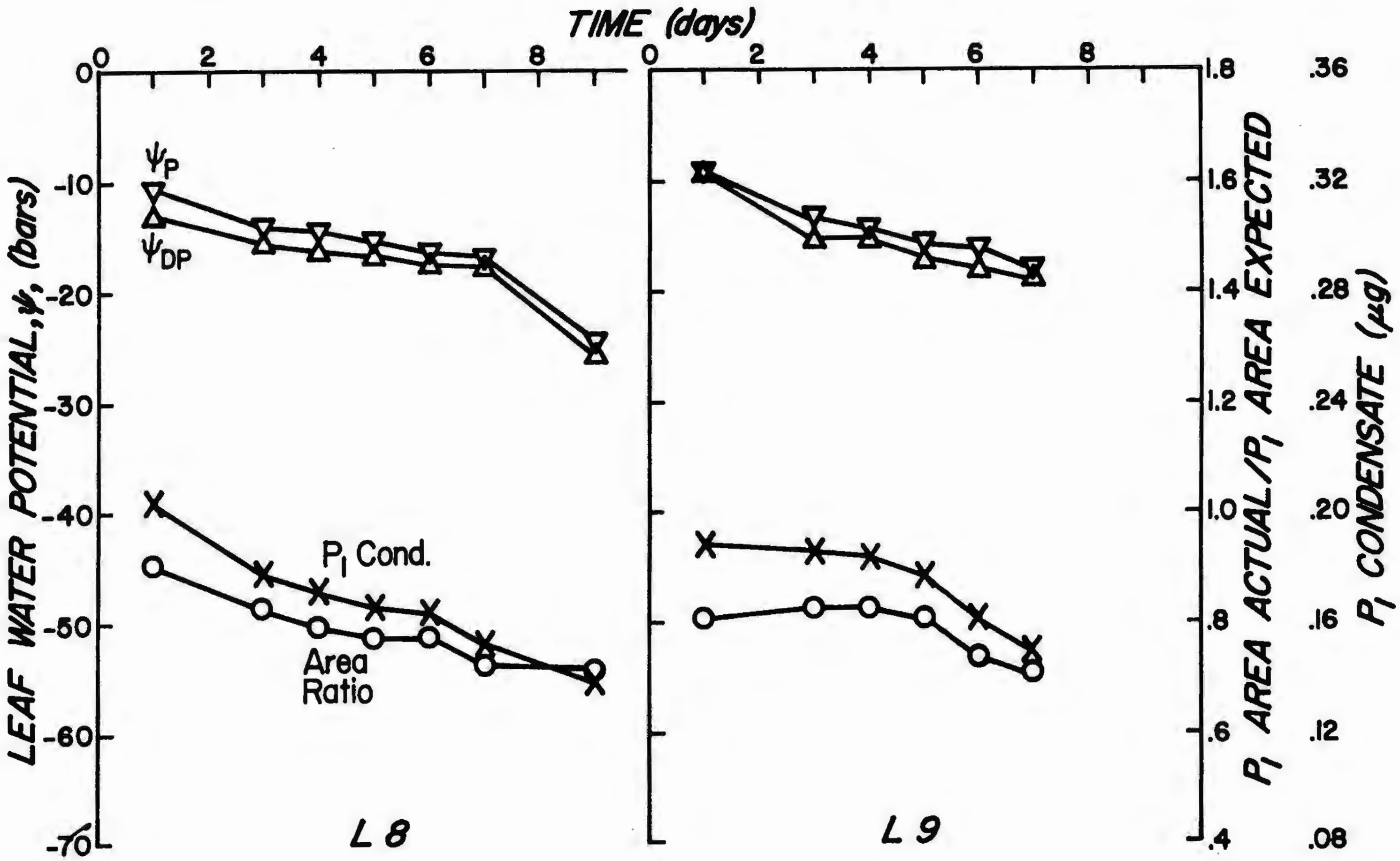


Figure 10. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Chlorophytum capense No. 1. L denotes Wescor leaf hygrometer.

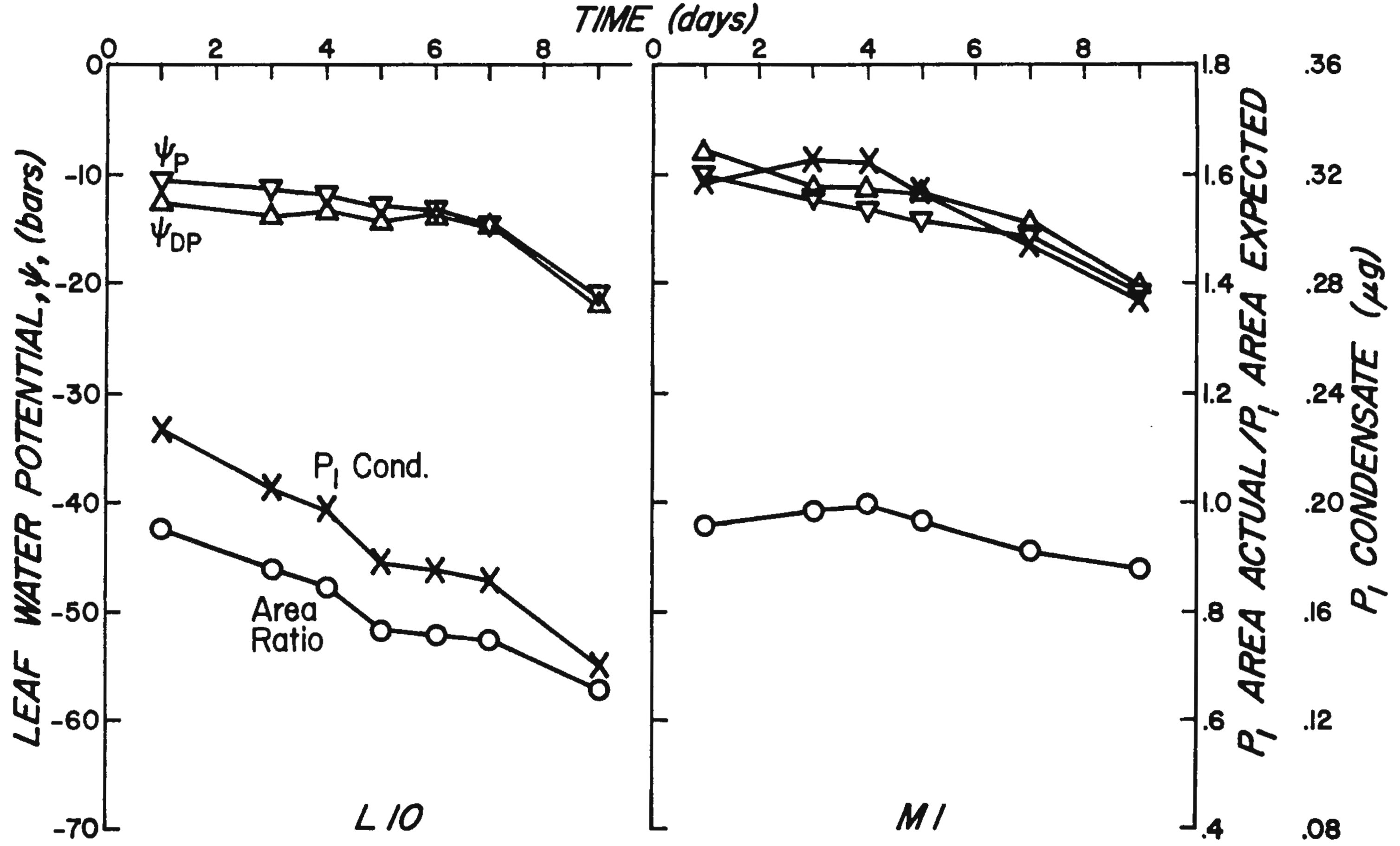


Figure 11. Relationship between time in days and leaf water potential (ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Chlorophytum capense No. 1. L or M denote Wescor or Merrill hygrometer, respectively.

Chlorophytum capense No. 2

Leaf water potentials determined in Wescor hygrometers were in close agreement (Figures 12 and 13). Lower water potentials were obtained in dewpoint mode than in the psychrometric mode and the difference was greater at the lowest water measured. The lowest water potential (-17 bars) determined in the Merrill unit was much higher than the lowest value obtained in Wescor leaf hygrometers and it was only 1 bar higher than a corresponding measurement done with the pressure bomb that same day. The differences in water potential obtained in both psychrometric and dew point mode in the Merrill hygrometer were not greater than 2 bars, but there was a tendency to lower water potentials in the psychrometric mode. Fluctuations in the volume of water condensed on the thermocouple do not correlate well with the changes in water potential in Wescor hygrometer measurements, but they do resemble those done with the Merrill hygrometer. Both types of hygrometers showed a decrease in volume of water condensed as water stress increased. The ratio P_1Aa/P_1Ae remained nearer 1 in the Merrill hygrometer than in the Wescor units. However, the decrease was not steady as the drought progressed.

Populus tremuloides

Water potential diminished rapidly from values ranging from -9.9 to -13.7 bars in the first day to values ranging from -22.4 to -36.8 bars according to measurements obtained with Wescor Units (Figure 14). Simultaneous water potential determinations monitored with the Merrill hygrometer indicated a decrease from -8 bars to aboue -40.6 bars. A general agreement in determinations made with both types of

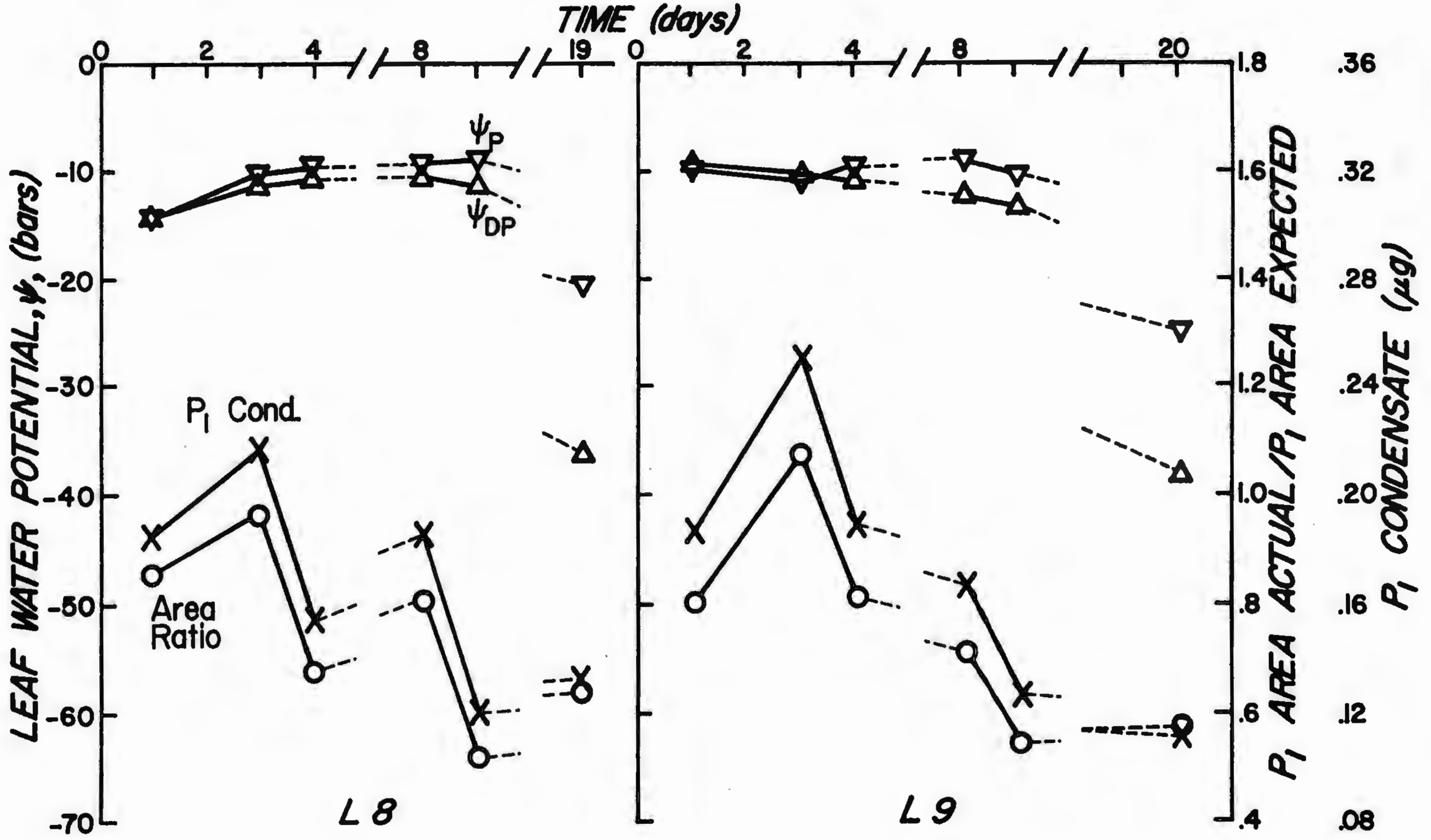


Figure 12. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Chlorophytum capense No. 2. L denotes Wescor hygrometer.

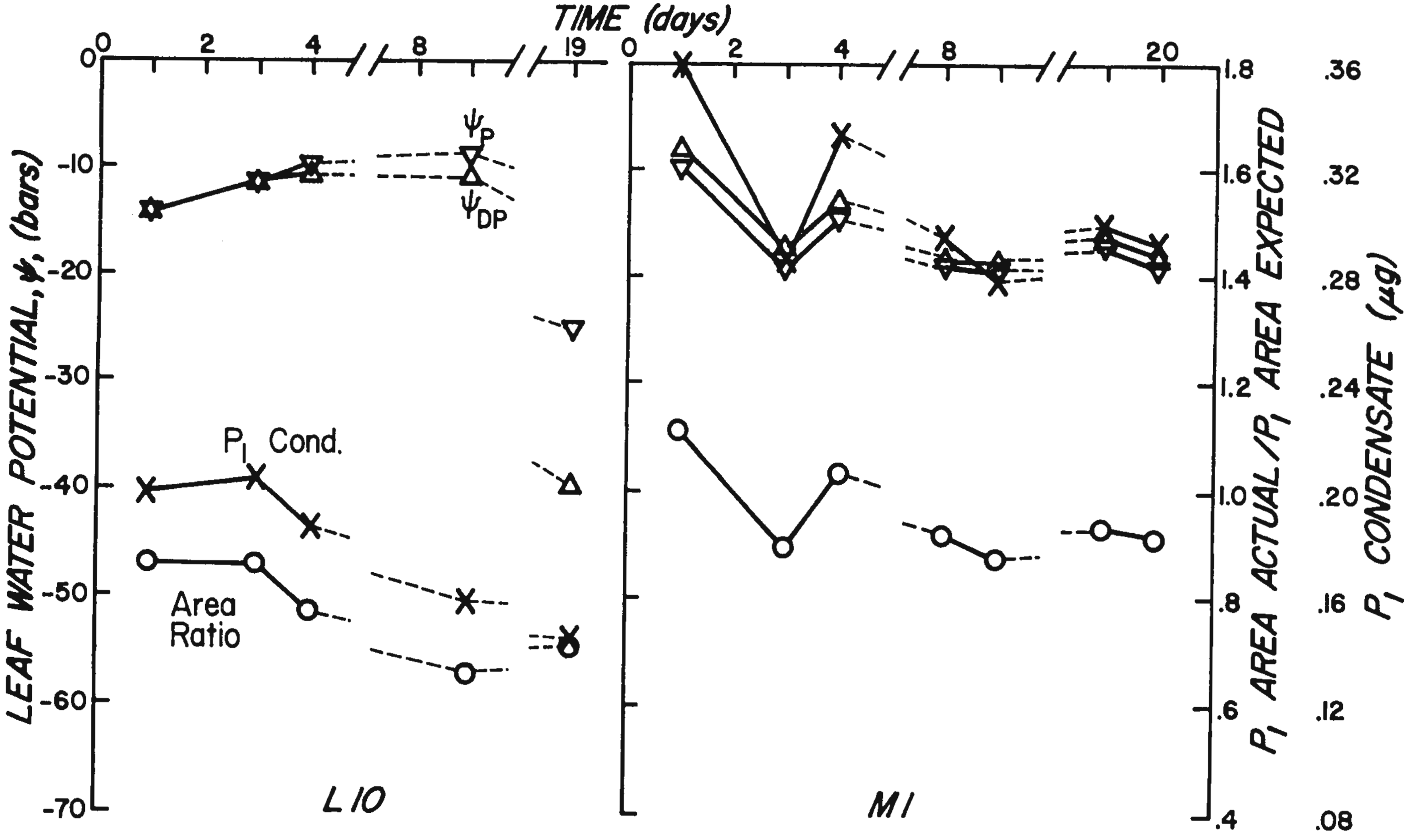


Figure 13. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and the dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Chlorophytum capense No. 2. L or M denote Wescor or Merrill hygrometer.

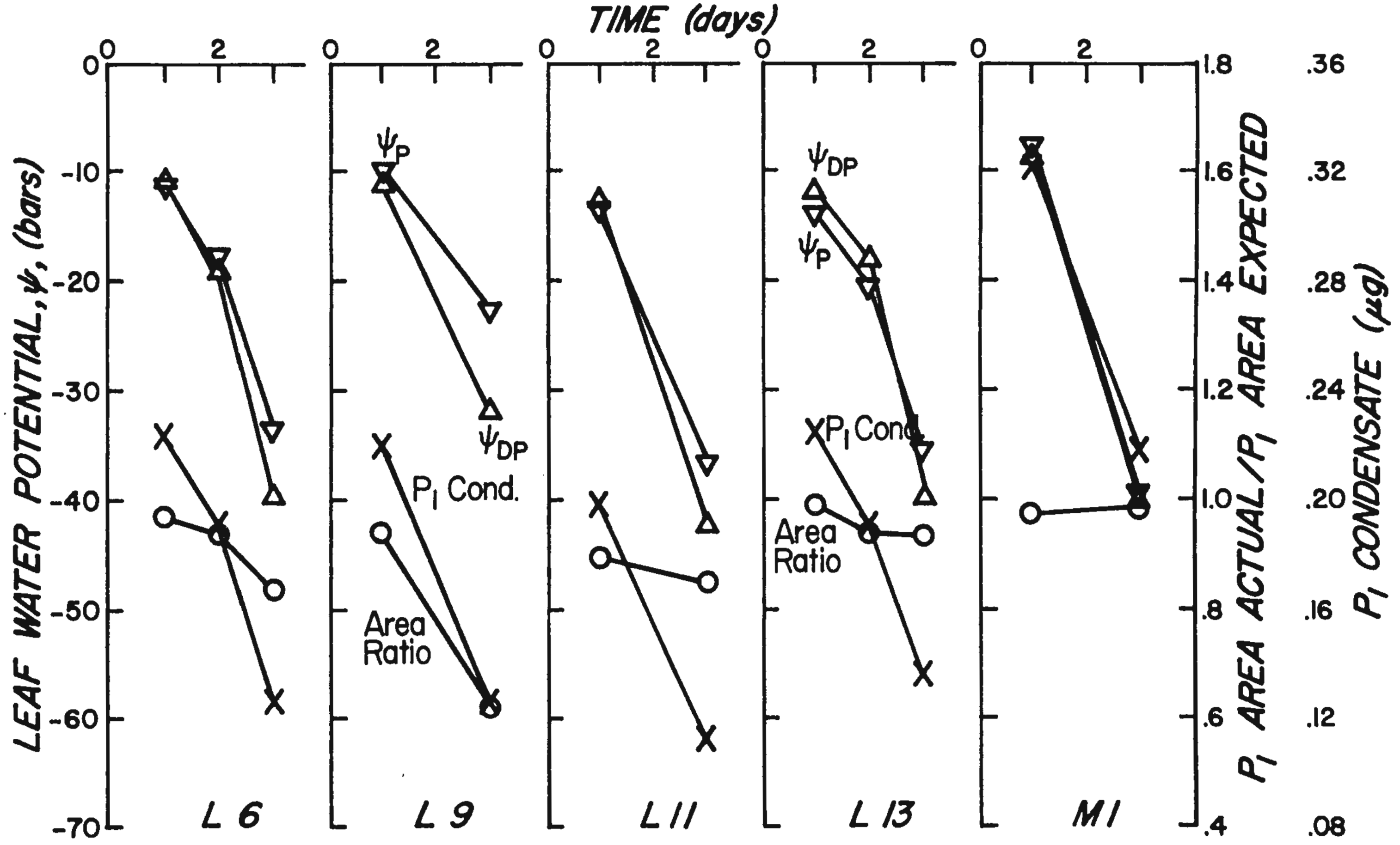


Figure 14. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Populus tremuloides. L or M denote Wescor or Merrill hygrometer, respectively.

hygrometers was obtained. Dewpoint readings indicated lower water potentials than those obtained from the psychrometric mode; the differences increased with increasing water stress, ranging on the last day from 4.1 to 9.1 bars in Wescor units and less than 1 bar in the Merrill unit. Variations in water volume condensed on the junction correlated well with the changes in leaf water potential; a decrement of about 43 percent was obtained in Wescor hygrometers and a corresponding decrease of 32 percent was measured in the Merrill unit. The P_1 area actually was closer to the P_1 area expected at the same water potential at the beginning, but decreased from 0.94 to 0.62 in L9.

As an effect of the temporary drought the plant lost all its leaves but new leaves developed a few days after rewatering.

Brassaia actinophylla

Poor agreement is observed among data obtained from the different hygrometer units (Figures 15 and 16). However, in L13 and Merrill hygrometer (M_1) fair agreement was obtained in water potentials measured in both psychrometric and dewpoint mode. In general, the resistance of the leaf tissue to cede water to the thermojunction, as measured by the ratio P_1Aa/P_1Ae, seemed to lower until the thirteenth day of the drying cycle. Although, data obtained from the thirteenth day suggested an apparent recovery in the water status, the plant continued progressively showing the drought effects in such a way that on the sixteenth day necrosis was observed in leaf veins of the entire plant, stem looked sunken and no additional readings could be obtained. The plant did not recover after rewatering.

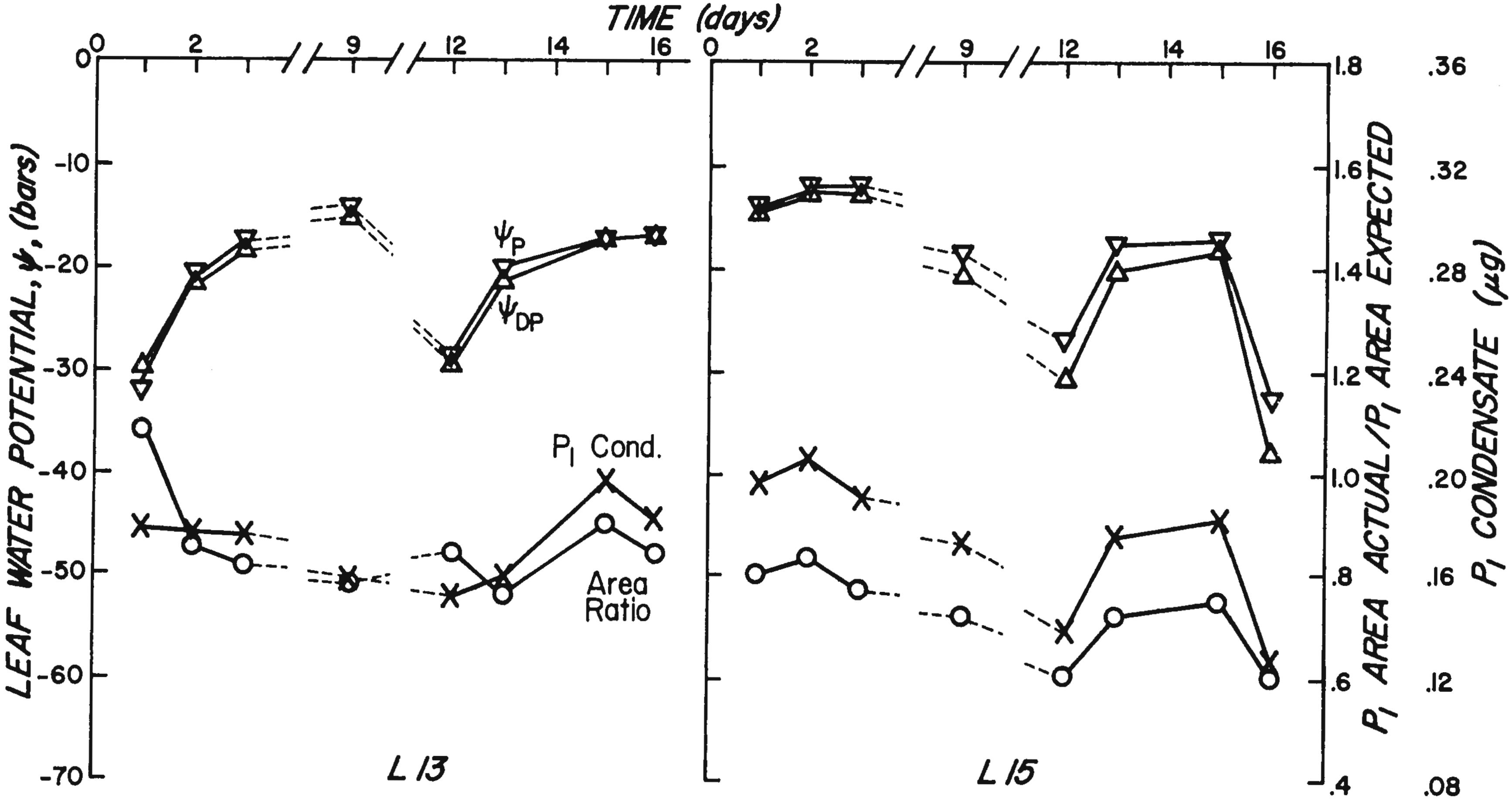


Figure 15. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Brassaia actinophylla. L denotes Wescor leaf hygrometer.

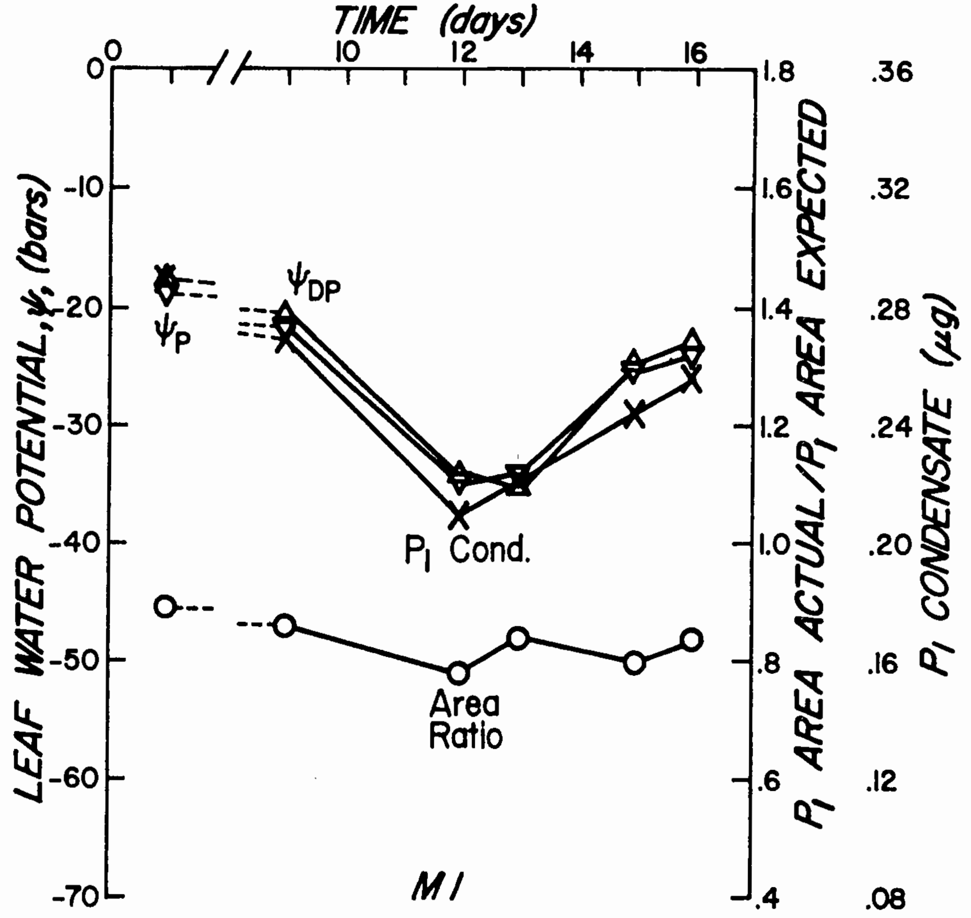


Figure 16. Relationship between time in days and leaf water potential (Ψ), measured in the psychrometric (ΨP) and dewpoint mode (ΨDP), ratio P_1 area actual/P_1 area expected and volume of water condensed on the thermocouple for Brassaia actinophylla. M denotes Merrill hygrometer.

Generalizations

After examining all the plant data some generalizations may be obtained:

1. The trend in leaf water potential (Ψ) as drought developed decreased, but with some variation among the different species. Populus, two Zea plants and Chlorophytum No. 1 showed a steady decrease in water potential throughout the drying cycle but Brassaia and to a lesser degree Chlorophytum No. 2 showed a variable behavior.

2. From psychrometric determinations of Wescor units plotted against simultaneous dewpoint determinations (Figure 17) it is apparent that the dewpoint consistently gives a lower estimate of the Ψ, and that this discrepancy progressively increases at lower Ψ values. A similar plot made with the water potential data obtained in Merrill units (Figure 18) shows that they are very close to the line of equality. The difference indicates that the water potential read in psychrometric mode is 0.66 bars lower than that in dewpoint mode. A statistical test used to compare regression lines (Neter and Wasserman, 1974) showed the lines fitting the data above indicated for each type of hygrometer (Figures 17 and 18) to be different (0.1 percent level of significance).

3. The trend in P_1 area, here used as a measurement of the volume of water condensed on the thermocouple, was variable as the water stress increased in the different plant species. Measurements done with Wescor hygrometers indicated steady decreases in the P_1/Aa as drought developed in both Zea plants, Chlorophytum No. 1 and Populus, but in Brassaia and Chlorophytum No. 2 a greater variability was obtained. Measurements obtained from Merrill hygrometers showed a consistent

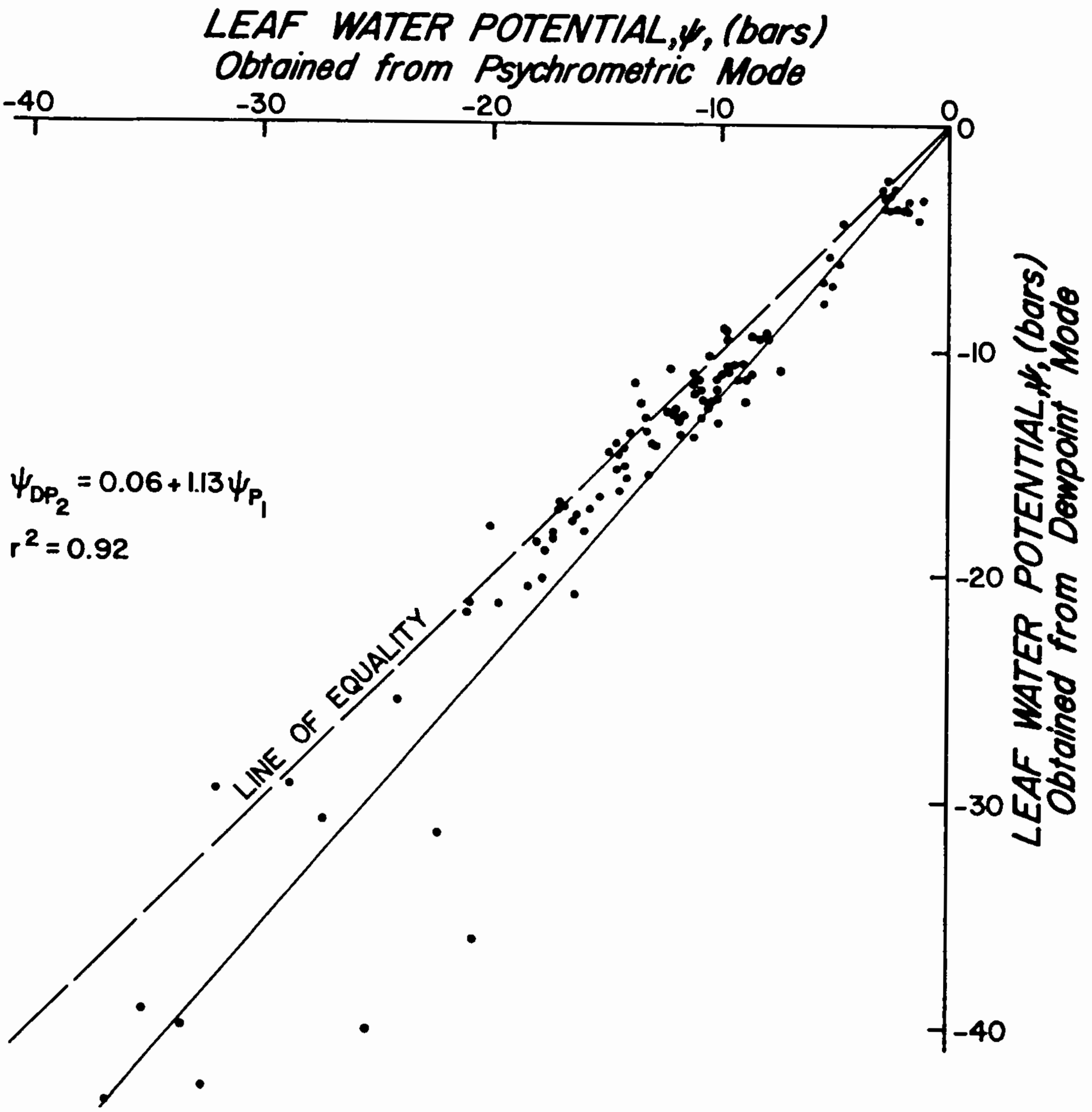


Figure 17. Relationship between leaf water potential obtained from psychrometric model (ΨP) and leaf water potential obtained from dewpoint mode (ΨDP) with Wescor leaf hygrometers; pooled data from all the plants

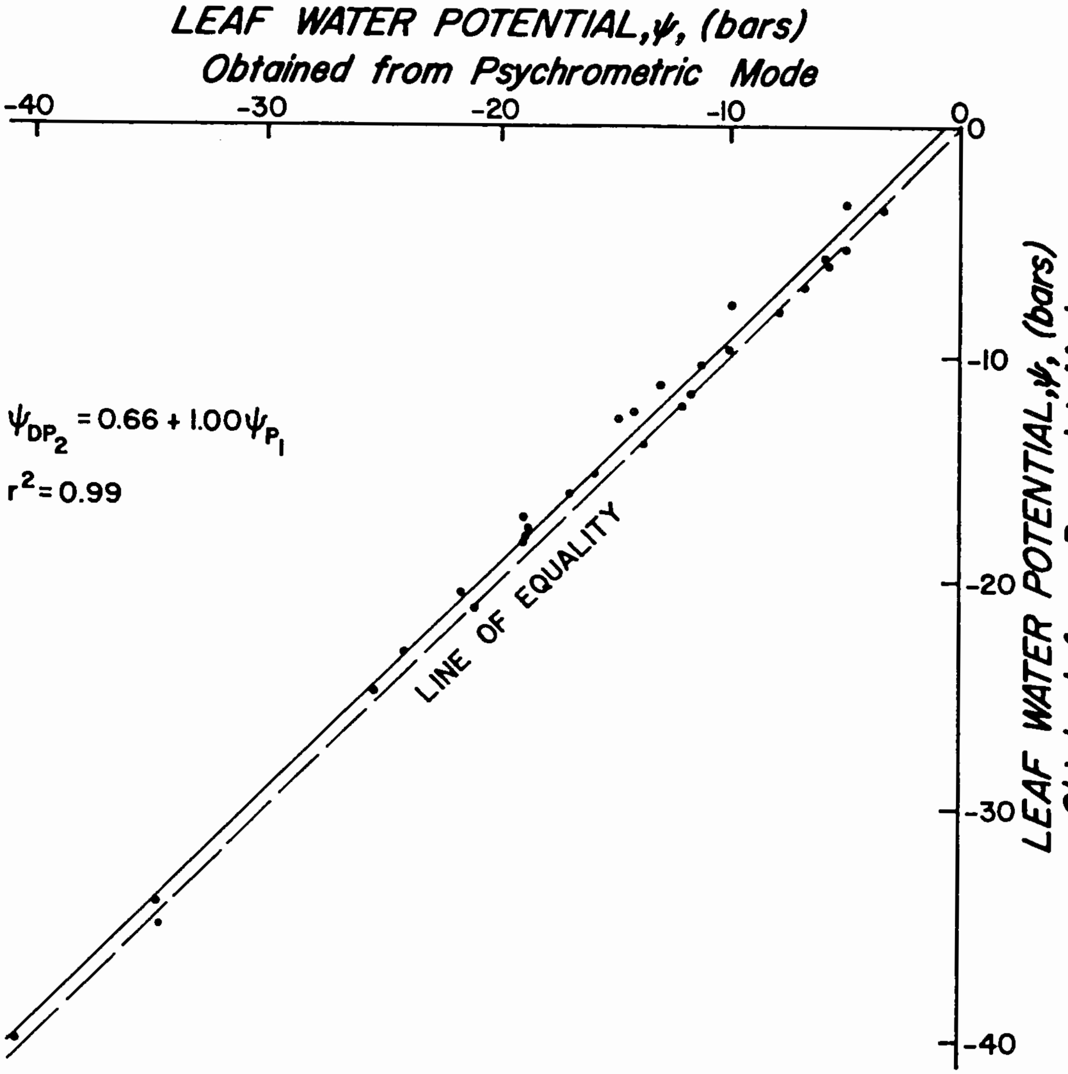


Figure 18. Relationship between leaf water potential obtained from psychrometric mode (ΨP) and leaf water potential obtained from dewpoint mode (ΨDP) with Merrill hygrometers; pooled data from all the plants.

decrease in actual P_1 area in all the plants studied as the water stress increased.

4. Plots of ratio P_1 area actual/P_1 area expected against Ψ using the data obtained with Wescor hygrometers on each plant (Figures 19 and 20) indicated a definite tendency for the ratio to decrease as water potential decreased in the two Zea plants, Chlorophytum No. 1 and Populus. No tendency was obtained in Chlorophytum No. 2 and Brassaia plants. A higher rate of decrease was obtained in Chlorophytum No. 1 than in Zea and Populus. A similar plot (Figure 21) with data obtained in the Merrill units shows a clear tendency of the P_1 area ratio to decrease at lower water potentials in Zea No. 2, the two Chlorophytum plants and Brassaia. Not enough data were obtained on Populus. The highest rate of decrease in P_1 area ratio was obtained in Chlorophytum No. 2; similar rates of decrease were obtained in Zea and Chlorophytum No. 1 and the lowest rate was measured in Brassaia. Since Chlorophytum No. 1 looked younger than the Chlorophytum No. 2, age differences may explain the different behaviors observed.

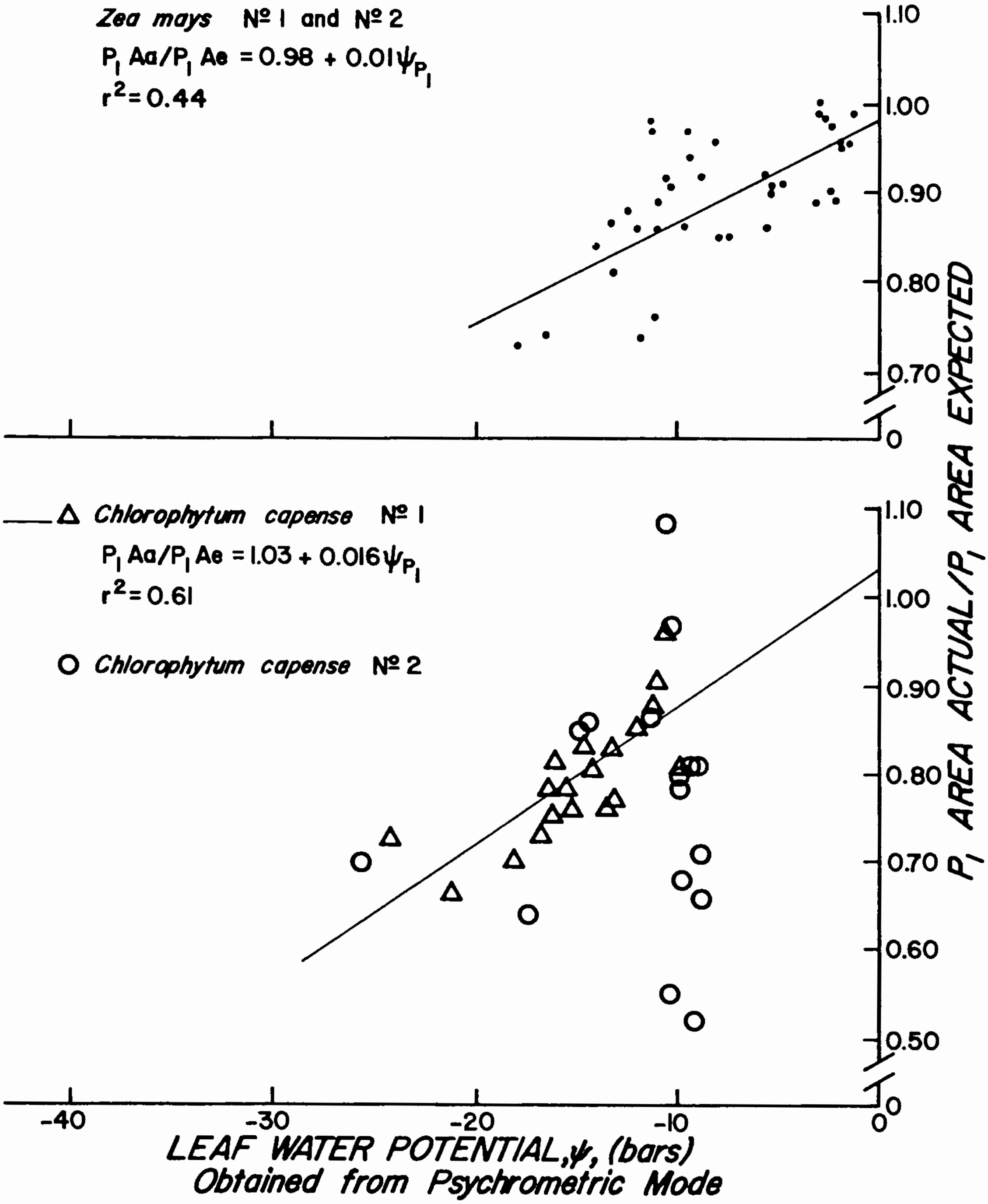


Figure 19. Relationship between the leaf water potential obtained from psychrometric mode (ψP) and the ratio P_1 area actual/P_1 area expected with Wescor leaf hygrometers for Zea mays and Chlorophytum capense plants.

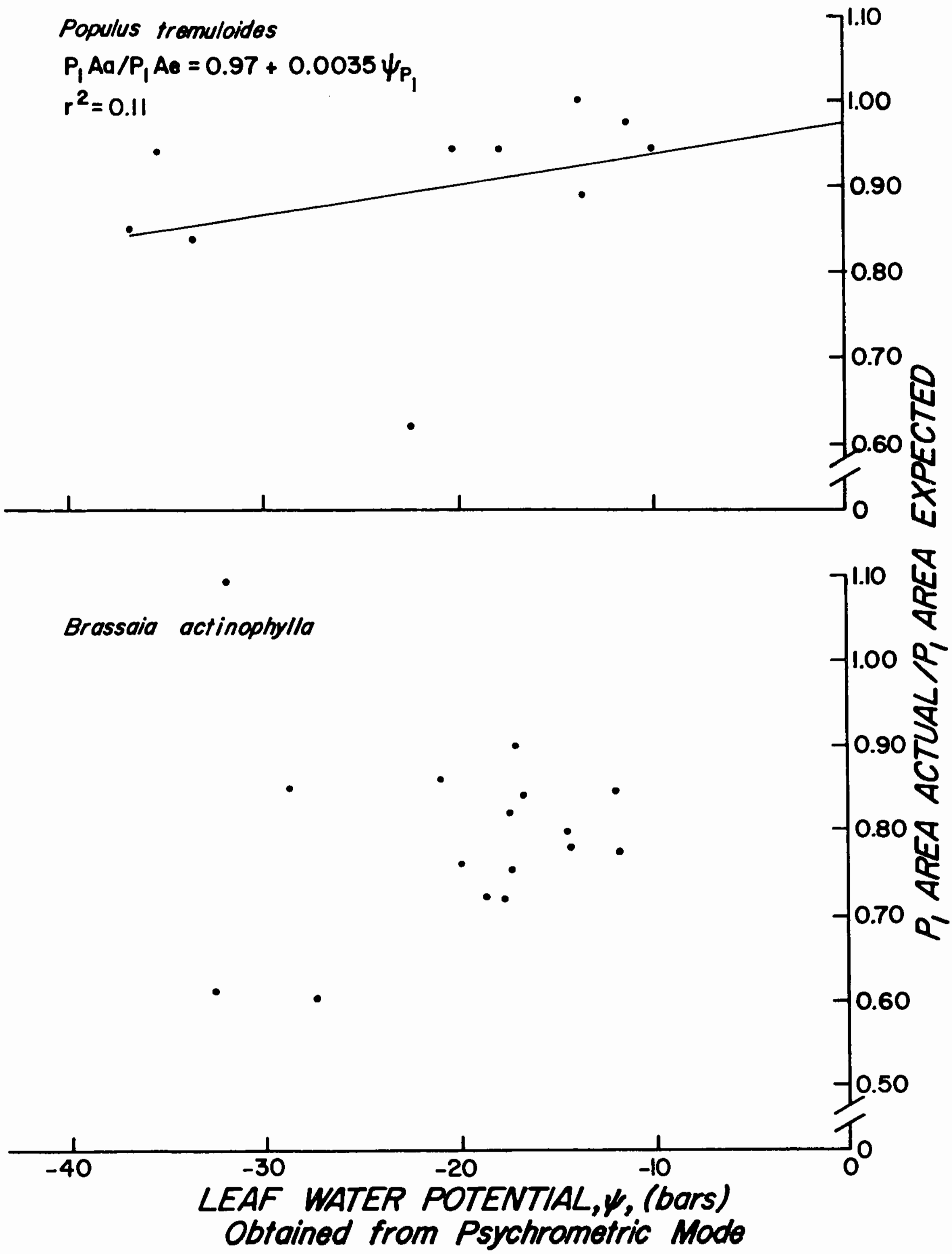


Figure 20. Relationship between the leaf water potential obtained from psychrometric mode (ΨP) and the ratio P_1 area actual/P_1 area expected with Wescor leaf hygrometers for Populus tremuloides and Brassaia actinophylla.

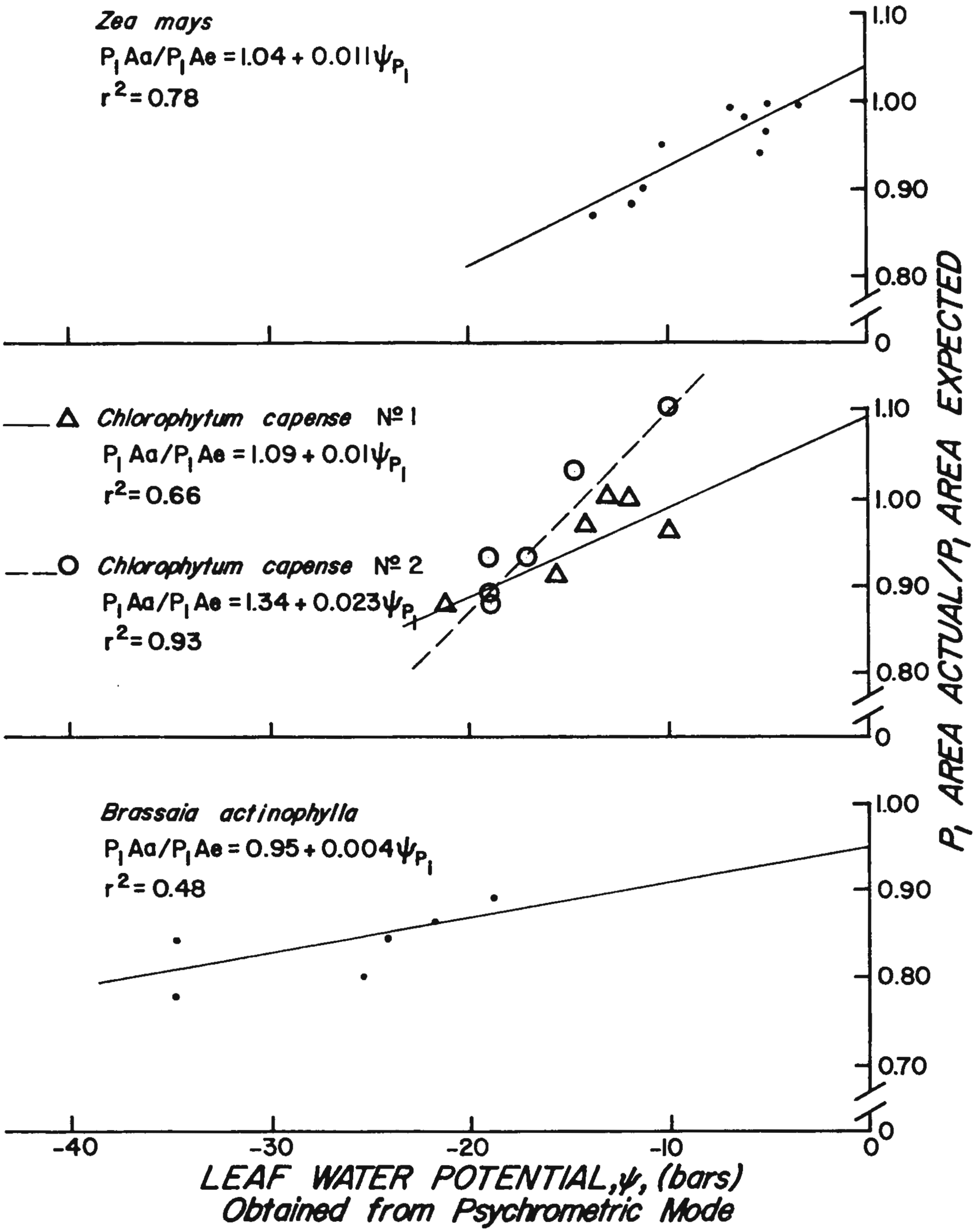


Figure 21. Relationship between leaf water potential obtained from psychrometric mode (ΨP) and ratio P_1 area actual/P_1 area expected with Merrill hygrometers for Zea mays No. 2, Chlorophytum capense and Populus tremuloides.

DISCUSSION

One of the main objectives of this study was to ascertain if the dewpoint method actually measures water potential under conditions of zero water movement, i.e. isopiestic conditions. There are several lines of evidence that such equilibrium occurred. First, water potential measurements on both standard solution and leaves in the dewpoint mode, remained steady for periods up to 300 seconds and even longer. Also, the area under psychrometric output P_2 recorded during evaporation phase after several minutes in the dewpoint phase was generally nearly equal to the psychrometric output P_1 (Figure 5). These support the conclusion that little net water evaporation or condensation was occurring from the wet junction during the dewpoint measurement. It was found, however, that the cooling coefficient for *in situ* leaf hygrometers needed to be lowered in reference to those found in dry air, as the plant water deficit increased to ensure the energy balance necessary to maintain the junction at dewpoint temperature. Theoretically, it is expected that the cooling coefficient, Π_v, once it has been determined for a given hygrometer should remain constant, but my own experience indicates that with lower relative humidity inside the chamber a lower temperature coefficient is necessary to set the junction more precisely at dewpoint temperature. It was easy to find the correct cooling coefficient and to match it in the circuitry, once this was done, the dewpoint might reflect the actual leaf water potential since the output could be read for a long time.

However, at the lowest water potentials, it was generally difficult to hold the junction at dewpoint temperature. I do not know if changes in sensitivity of the thermojunction can be introduced by substantial changes of the cooling coefficient from the value obtained in dry chamber.

Comparison of Dewpoint and Psychrometric Determinations of Leaf Water Potential

When water potential was measured in both the psychrometric and dewpoint mode with Wescor units, lower water potentials were found in dewpoint mode than in the psychrometric mode with a definite trend to increase the discrepancy at lower water potentials (Figure 17). Merrill hygrometer data indicated 0.66 bar lower when the water potential was determined in the psychrometric mode than in the dewpoint mode (Figure 18). The differences in leaf surface area exposed to the junction may have contributed to the different behavior of the two types of units. The leaf surface area in Merrill hygrometer is about 10x that of the Wescor unit which means that a lesser total leaf resistance will influence the psychrometric water potential determination in the Merrill unit. It may well be for that reason that psychrometric and dewpoint determinations agreed closer even at lower water potentials.

When the temperature of the junction in a hygrometer is depressed by Peltier cooling, water vapor and heat will start to flow into it from the surrounding environment, this process decreases chamber vapor pressure. When cooling ceases water will start to evaporate from the wet junction and the chamber has higher vapor pressure than the mesophyll.

If leaf resistance is high a psychrometric reading made at 5 seconds, when the output becomes level, has enough time to bring the chamber vapor pressure above leaf vapor pressure. The greater the leaf resistance the greater the difference in humidity between the chamber and the mesophyll spaces (Wiebe and Prosser, 1977). As a result water potential monitored in the psychrometric mode may be erroneously too high. Since little water moves during the dewpoint mode, resistance is not limiting and the determination might be expected to be more accurate.

When leaf resistance is sufficiently high to seriously restrict water movement, an additional error may be involved in the psychrometric determination. Since leaf water movement is limited, a smaller volume of water will be condensed on the thermocouple by the cooling current, when it ceases water evaporation will initiate at a higher rate, shortening the psychrometric reading time. In such situations to have a steady output as that obtained in dewpoint mode would increase the accuracy in the water potential measurement (Figure 5). The regression line obtained with pooled data of Wescor units in which the differences between water potential determined in psychrometric and that on the dewpoint mode was smaller at high water potentials and increased toward the lower water potentials measured are evidence of such behavior.

If the leaf water potential measured in the dewpoint mode reflects the true water potential, it could be speculated that in the Merrill hygrometers the psychrometric reading underestimated the true water potential of the leaf by a constant value (0.66 bars) and in the Wescor units the psychrometric reading overestimated the true water potential in a larger value that, moreover, increased the water potential decreased.

Influence of Leaf Water Potential on Amount of Water Condensed

Measurements of water volume condensed on the thermocouple over standard solutions demonstrated decreasing water condensation at lower water potentials. Data obtained with plants revealed the decrease in volume of water condensed on the junction was even greater than the decrease obtained on standard solutions. This was reflected in decreases of ratio P_1A actual: P_1A expected, in most cases, going farther below to 1.00 as water stress increased.

An analysis of the Figures 19, 20, and 21 shows ratio P_1 area decreases with decreasing water potential were variable among plants and also in measurements done on the same plant with the two type of units. If the volume of water condensed on the juntion were only a function of the water potential of the leaf, the ratio P_1 area should have been near 1.00 throughout the drying period but, the decreases observed and the variability among plant species suggested that an additional factor other than the water potential influences the actual area under the psychrometric reading. Or said in another way, some other factor influences the amount of water condensed on the junction. The interpretation is that such decrease is the expression of the increasing leaf resistance as the stomates close in response to progressive plant water stress. Differences among species could also be a function of different leaf resistances. Inasmuch as leaf

leaf resistance increased and consequently less water was transferred to the junction at lower water potentials, the reliability of the leaf water potential determination in the psychrometric readout was more limited than those determinations in the dewpoint mode in which isopiestic conditions could be reached.

CONCLUSIONS

In situ monitoring of leaf water potential in the psychrometric mode gives an erroneously high estimate of leaf water potential when water vapor diffusion through the leaf is limited. This error is attributed to the fact that the leaf surface area exposed to free junction in an *in situ* leaf hygrometer is small, and it is not found in sample chambers which enclose larger leaf surfaces. The error becomes progressively higher at high leaf resistances. Because significant errors in leaf water potential could result from severe thermal gradients if the leaf area sampled in an *in situ* leaf hygrometer is increased, such area must be kept small. Under conditions of high leaf resistance and small sampling area the dewpoint method is preferred to the psychrometric mode for more accurate leaf water potential measurements.

If a large area surface of the leaf can be used both the psychrometric and the dewpoint methods can be used interchangeably without obtaining significant differences.

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