INTERNAL REPORT 3

DEVELOPMENT OF CUVETTE EQUIPMENT

David J. Salo, John A. Ringo, James H. Nishitani, and
Richard B. Walker

INTRODUCTION

The cuvette technique has long been used in conducting CO_2 assimilation studies. However, because temperature control in plastic enclosures has been difficult to maintain, the method has often been subject to criticism. Recently, Siemens Corporation designed, and now manufactures, a reliable temperature and humidity controlled system whose utility and flexibility have been demonstrated by Schulze (1970). Though a Siemens-Koch cuvette system is currently available at the University of Washington, its use in "factors studies" precludes its use for routine CO_2 monitoring. Further, accessibility to a single unit prevents duplicate sampling during a given period. As a result, the primary 1970-1971 Biome effort in this laboratory was on design, fabrication, and testing of an inexpensive temperature-controlled cuvette system as well as the collection of preliminary field data.

EQUIPMENT DEVELOPMENT

Cuvette

Two 7-liter chambers (Figure 1), approximately 29 x 22 x 11 cm, were constructed of 9.5-mm Plexiglas and were provided with 3.2-mm aluminum bases. The removable cover of each unit is sealed with a 3.2-mm rubber gasket and is secured with 20 wing nuts and bolts. Each cuvette is equipped with a small 0- to 6-V, 0.15- to 1.5-amp dc fan with a 10-cm blade for mixing air to prevent boundary layer and temperature gradient buildups.

The aluminum floor of each chamber is securely bolted with five screws to the cold plate of an 80-W Cambion model 7250-1 "forced convection thermoelectric assembly" to maximize contact between the two energy exchange surfaces. In addition, various heat sink designs with good thermal bonding potential are being evaluated to determine the relative effectiveness of each in increasing floor surface area and system cooling capability. The most effective device will ultimately be incorporated into the chamber design.

Power Supply--Controller

Each model 7250-1 cooler operates in a range between 0 and 18 V and 0 and 6 amp, and has a cooling capacity of 272 Btu/hour. A power supply-temperature controller-fan controller (Figures 2, 3) was designed to provide a variable-current output in the cooler operating range as well as a voltage source for two variable-output cuvette fan controllers.

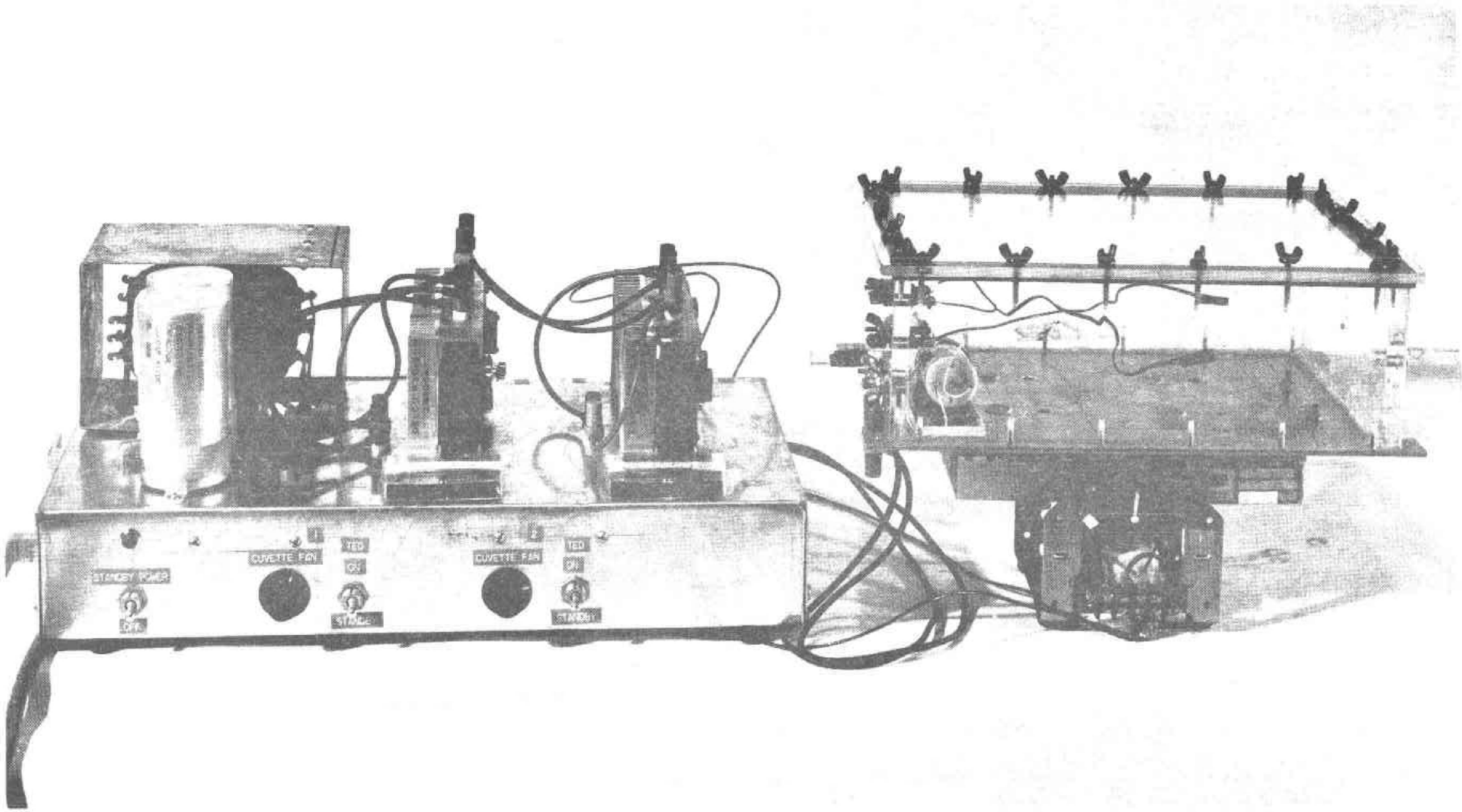


Figure 1. Cuvette and Power Supply - Temperature Controller - Fan Controller

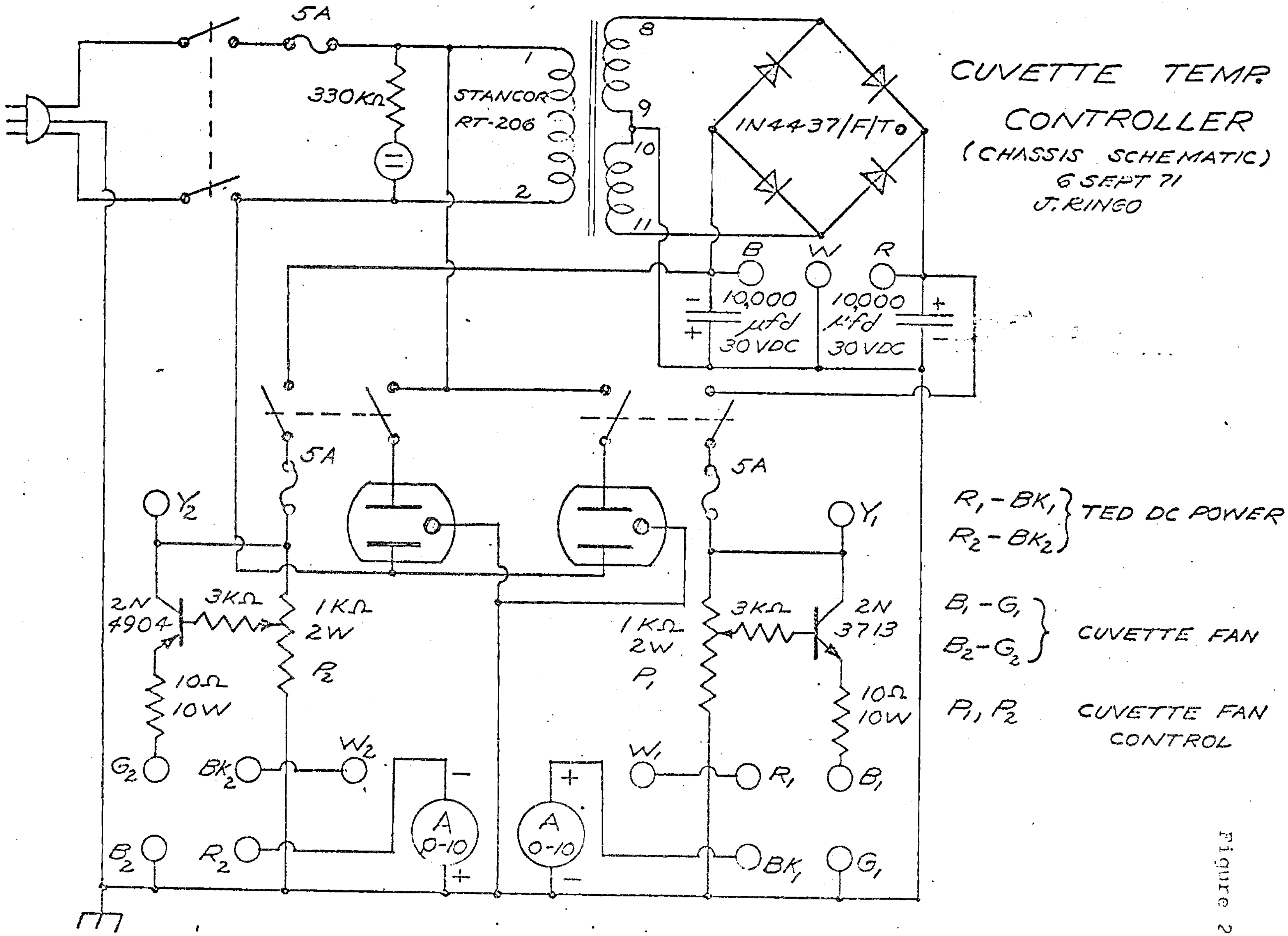
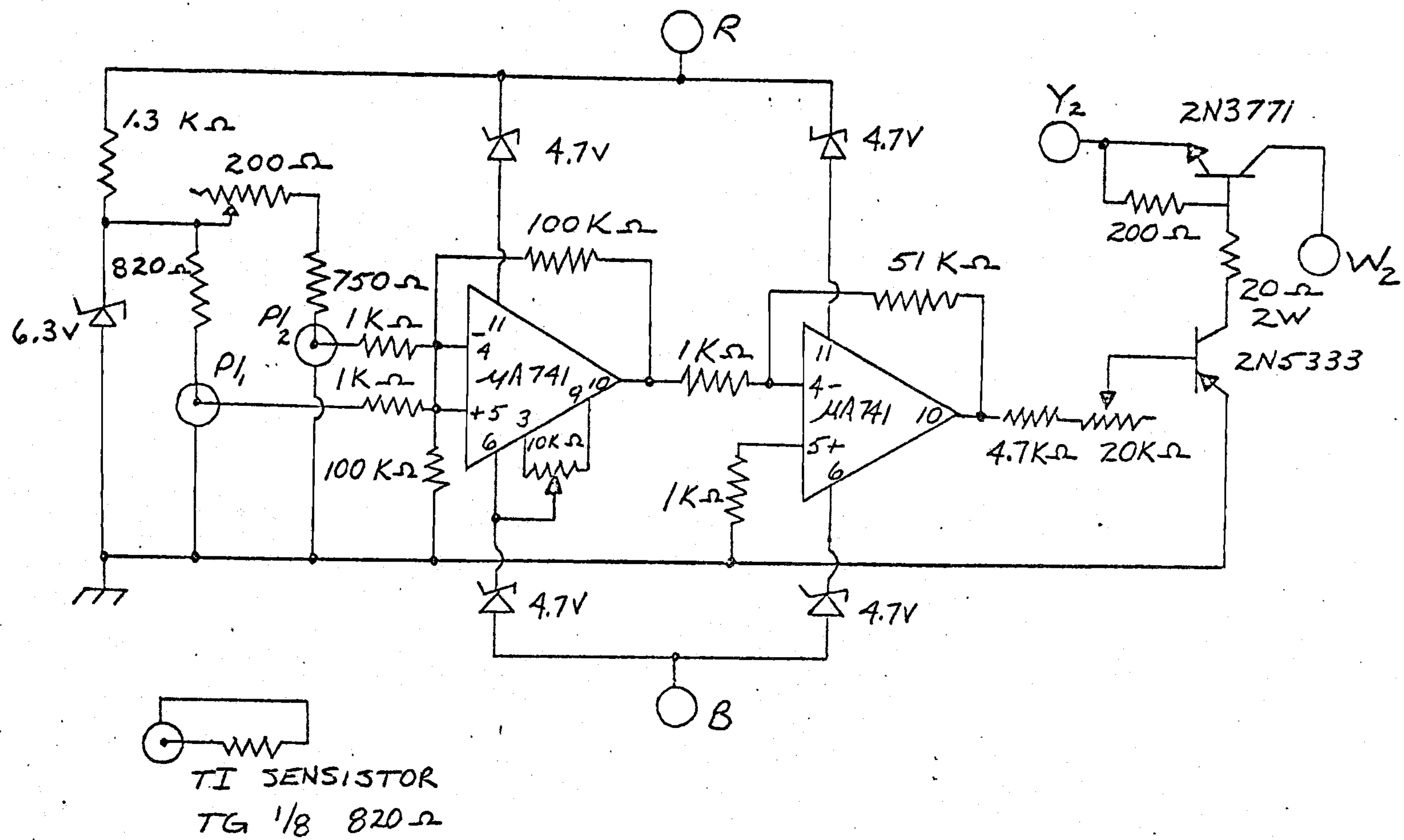

CUVETTE TEMP. CONTROLLER
(CHASSIS SCHEMATIC)
6 SEPT 71
J. RINGO
5A
330KΩ
STANCOR RT-206
1
2
8
9
10
11
1N4437/F/T
B
W
R
10,000 μfd 30 VDC
10,000 μfd 30 VDC
5A
5A
Y2
Y1
2N 4904
3KΩ
1KΩ 2W
P2
10Ω 10W
3KΩ
1KΩ 2W
P1
2N 3713
10Ω 10W
G2
BK2
W2
W1
R1
B1
B2
R2
A 0-10
A 0-10
BK1
G1
R1 - BK1
R2 - BK2
TED DC POWER
B1 - G1
B2 - G2
CUVETTE FAN
P1, P2
CUVETTE FAN CONTROL

Figure 2.



CONTROL SCHEMATIC
6 Sept. 1971

Figure 3

The power supply, which can be used to operate two temperature controllers, consists of a Stancor RT-206 rectifier transformer, a diode bridge rectifier (1N4437/F/T), and two 10,000-μ capacitors and provides an output of approximately 22 V. The design is standard and is similar to that illustrated in such elementary electronics texts as Hickey and Villines (p. 438).

Each of the two cooler-control circuits was developed around a sensitor (TG 1/8, 820Ω--Texas Instruments) bridge, the unbalance in which (ΔV) is amplified by two μ741 integratedcircuits. The amplified ΔV is then converted (2N5333, 2N3771) to a variable-current source (0 to 4.5 amp) to power the thermoelectric device when a temperature differential occurs between cuvette and external environments.

Performance

Originally, diodes formed from transistors were used as temperature sensing devices. Because of their mass, however, these units had slow response times and have been replaced by the smaller sensistors. Though evidence of both good response time and potential control capability is available for these sensors, tests are continuing to determine their specific effect, and that of increased floor surface area, on cuvette cooling capacity.

The only field performance data collected for the system were obtained during the summer of 1971 using diode sensors. Except on one occasion, net radiation did not exceed approximately 0.8 cal cm^{-2} min^{-1} and cuvette temperature was maintained within ±0.75°C of ambient. On 3 August (1230 to 1330 hours, PST), when values between 0.95 and 0.98 cal cm^{-2} min^{-1} were recorded, the internal cuvette temperature at leaf level was maintained with ±0.25° to 1.0°C of ambient. (Occasionally, unexplainable cuvette-to-ambient differences as great as 2.0° to 2.5°C have occurred briefly but should be prevented by using sensistors and heat sinks.)

Cost

In addition to its simplicity, ease of handling, and cooling capability, the system described here is relatively inexpensive. Total cost of two thermoelectric coolers ($540), two cuvettes ($40), and a control package ($90) was approximately $670.

FIELD RESEARCH IN CARBON DIOXIDE ASSIMILATION

David J. Salo, Richard B. Walker, and Craig Amundsen

Field trials for the system were conducted at the Thompson Research Center during July and August. Results presented here are considered representative of those obtained during the study period and include data collected during both clear and overcast days. Night respiration results have also been included.

System

The system utilized (Figure 4) included an air intake approximately 25 m above the ground on a tower located 3 m from that used as an access to study trees. Air was drawn to the ground through a 20-liter "mixing reservoir" to assure uniformity and prevent "noisy" analyzer output, and was then passed through a manifold for distribution to two Reciprotor pumps. One of these pumped a comparison airstream to the URAS II infrared gas analyzer housed 46 m away in the permanent site building, and the other moved air to the cuvette located at 17.2 m back to the ground, through a flowmeter, and then to the differential analyzer. The attenuating reservoir, manifold, pumps, and flowmeter were located in a shelter at the tower base as was the power supply-temperature controller-fan controller. A Honeywell recorder and the URAS II, however, were located in the site building. Two 0.1-mm copper-constantan thermocouples were used for temperature sensing inside the cuvette and one 22-gauge thermocouple was positioned in a shaded location outside.

In addition to cuvette data, meteorological data were provided by instruments located on the adjacent tower. Scholander bomb and pressure infiltrometer samples were routinely taken and correlated with cuvette and meteorological data during all trials. Sampling was carried out at cuvette level and values reported here represent averages --two to three twigs for P_s and five to seven needles for P_{stom} (stomatal infiltration pressure)--in each case.

Results

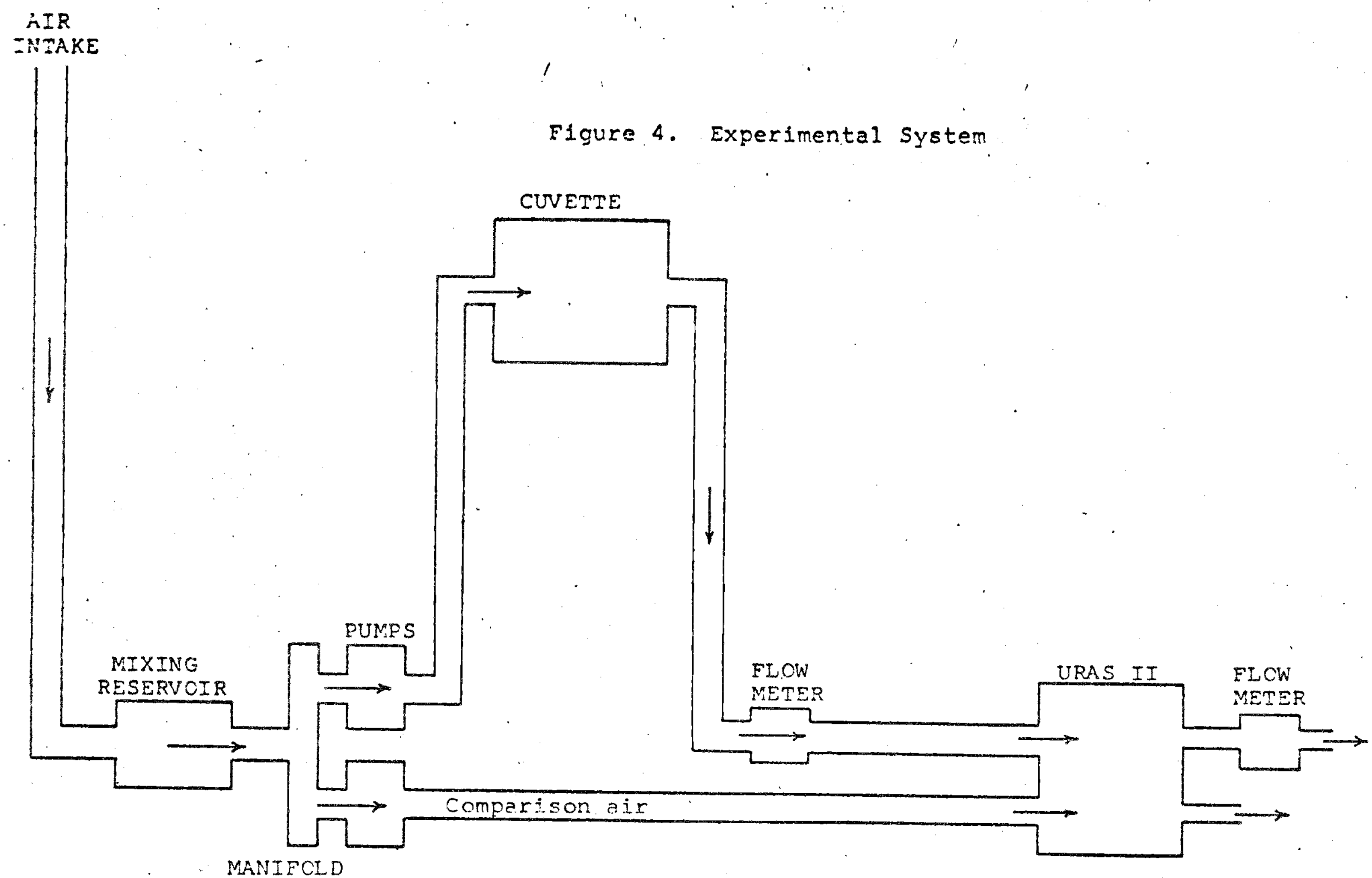
Summer data obtained agree with those of other investigators in suggesting a close relationship between assimilation, stress, and stomatal behavior. To illustrate this, results obtained on two representative days, one clear (3 August) and the other overcast (23 July), are briefly discussed below, and suggest that stress operates in at least two ways to reduce net assimilation.

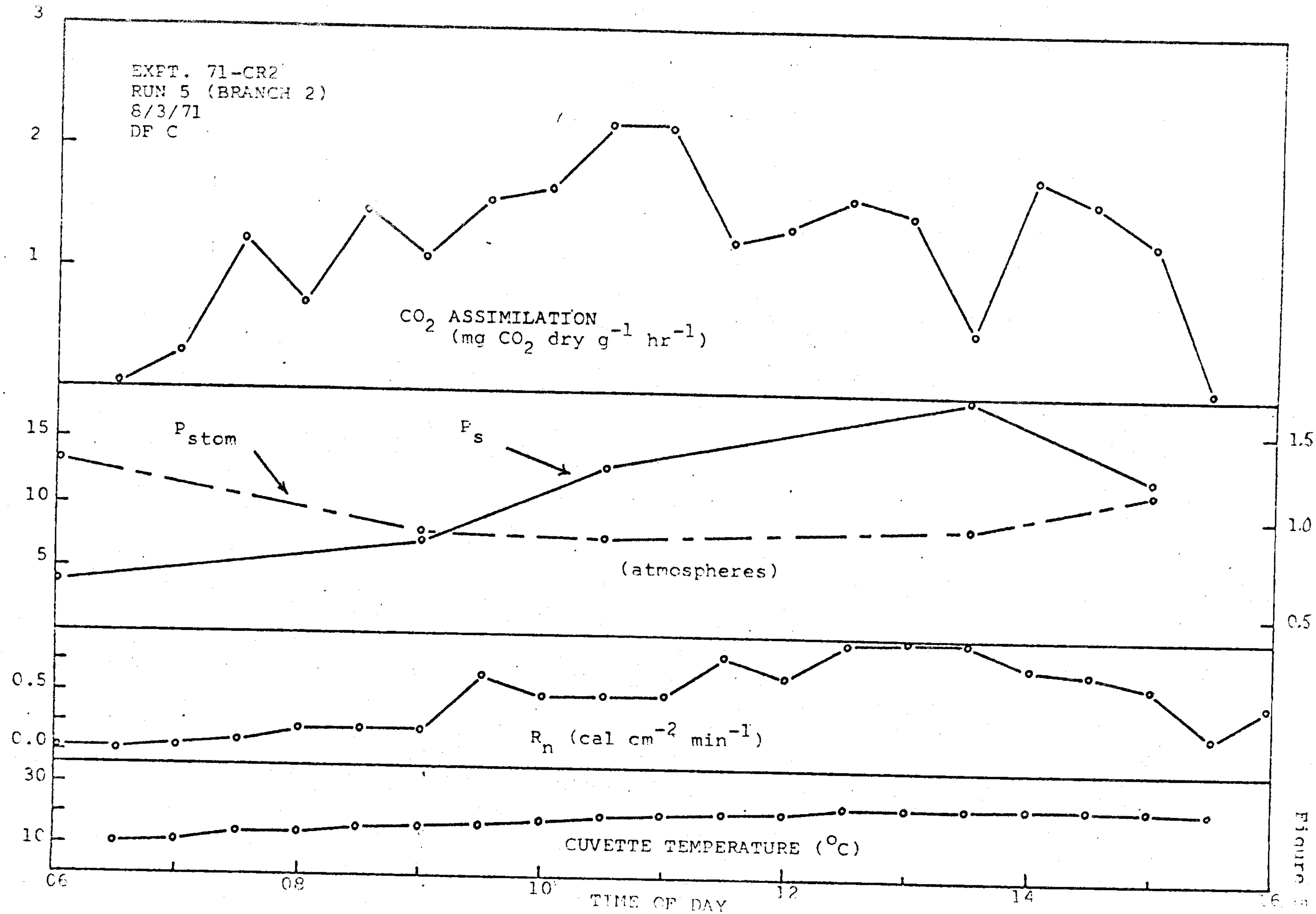
In addition to a direct effect, it is believed that when stomatal aperture is less than 50% of maximum (P stom $\geqq$ 15 psi), gas exchange is resisted to such an extent that net assimilation is greatly reduced. At the same time transpiration loss is also markedly reduced, resulting in increased ψ.

The direct effect of stress on photosynthesis appears to be a likely reason for low assimilation at 1330 on 3 August (Figure 5). At this time, P_s reached approximately 19 atm and assimilation had dropped to one-fourth of the high value for the day, even though stomatal aperture was essentially the same as at 0900 hours and R_n was 0.97 ly min^{-1}. The effect of stomatal closure was obvious subsequently, for as P_{stom} increased from 14 to 17 psi, P_s dropped 6.6 atm and, after a brief recovery, net assimilation fell to zero by 1530 hours.

In general, on all clear days sampled, light did not appear to limit photosynthesis; maximum assimilation rates occurred between 1030 and 1130 hours; CO_2 uptake decreased between 1130 and 1230 but was accompanied by a continuous stress increase and only small changes

Figure 4. Experimental System




EXPT. 71-CR2
RUN 5 (BRANCH 2)
8/3/71
DF C
CO_2 ASSIMILATION
(mg CO_2 dry g^{-1} hr^{-1})
P_{stom}
P_s
(atmospheres)
R_n (cal cm^{-2} min^{-1})
CUVETTE TEMPERATURE (°C)
TIME OF DAY
3
2
1
15
10
5
1.5
1.0
0.5
0.5
0.0
30
10
06
08
10
12
14
16

Figure

in stomatal aperture; varying degrees of afternoon stomatal closure followed and coincided with further depression of assimilation and relief of stress.

On 23 July (Figure 6), which was overcast and cool, the pattern of net assimilation P_s, and P_{stom} differed markedly from that observed under fair conditions. In this situation, it is apparent that light was limiting during morning hours as assimilation rates responded to R_n changes during this period. As R_n increased from 0.1 ly min^{-1} at 0830 to 0.2 ly min^{-1} at 1230, assimilation rate increased 2.8 times and was 2.66 mg CO_2 dry g^{-1} hr^{-1}, or 89% of the sampling period high, which was recorded on 27 July when R_n was 0.80 ly min^{-1}.

In addition to radiation, other striking differences between cloudy and clear days existed. For example, P_s did not exceed 11 atm on 23 July, but on 3 August P_s values were as high as 19 atm. Stomatal infiltration pressures had increased noticeably between 1330 and 1530 hours on 3 August but on 23 July, P_{stom} remained at approximately 0.8 atm, indicating that stomatal closure did not occur. It is likely, though, that stress increased enough to reduce photosynthesis in the afternoon, as assimilation rates had begun to drop by 1330, though T_n remained stable at approximately 0.2 ly min^{-1}.

The high assimilation rates observed here under overcast conditions are similar to those obtained by Walker and Salo (1970-1971) under controlled environment conditions (Figure 7) and by Walker (Figure 8), working with trees in the greenhouse garden during cool, low-radiation days, and suggest high photosynthetic rates when cool conditions exist. It is likely that during warm, clear days water stress limits photosynthesis markedly and often results in a complete cessation during afternoon hours. Because this does not occur under overcast conditions, cloudy, cool-day photosynthetic rates are often high.

Summary

Though it is impossible to reach definite conclusions from a limited amount of data, some general comparisons between clear and cloudy days in July and August 1971 have been tabulated below.

	clear		overcast
1.	Light did not limit net assimilation.	1.	Light limited net assimilation.
2.	Maximum assimilation rates occurred between 1030 and 1130 hours.	2.	Time of day for maximum assimilation was unpredictable but rates could exceed maxima for sunny days.
3.	Photosynthesis began to decrease by 1130 to 1230 hours.	3.	Photosynthesis decreased during the afternoon but time varied.
4.	Assimilation decreased before P_s decreased and P_{stom} increased.	4.	P_s did not decrease nor did P_{stom} increase during sampling periods.

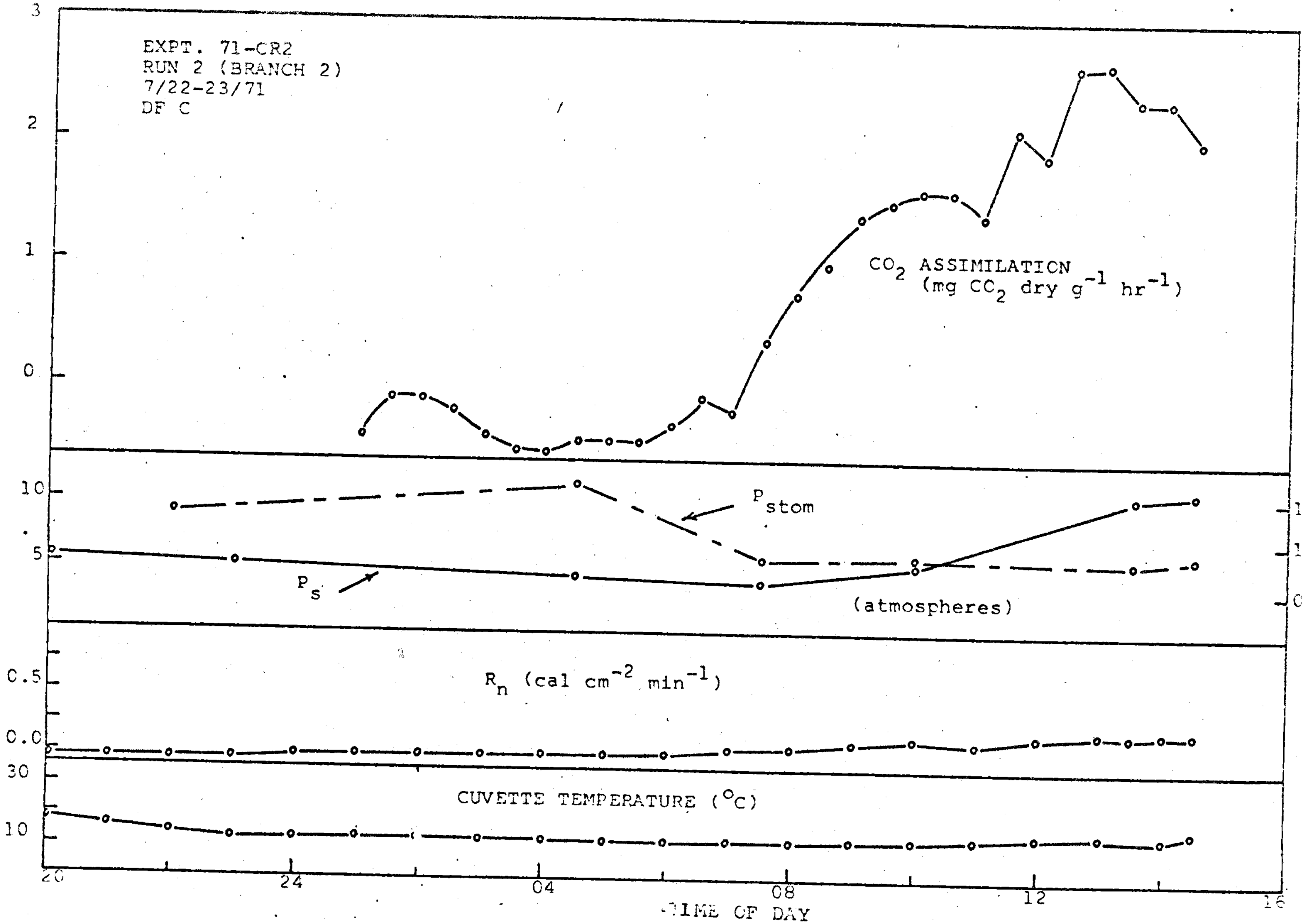
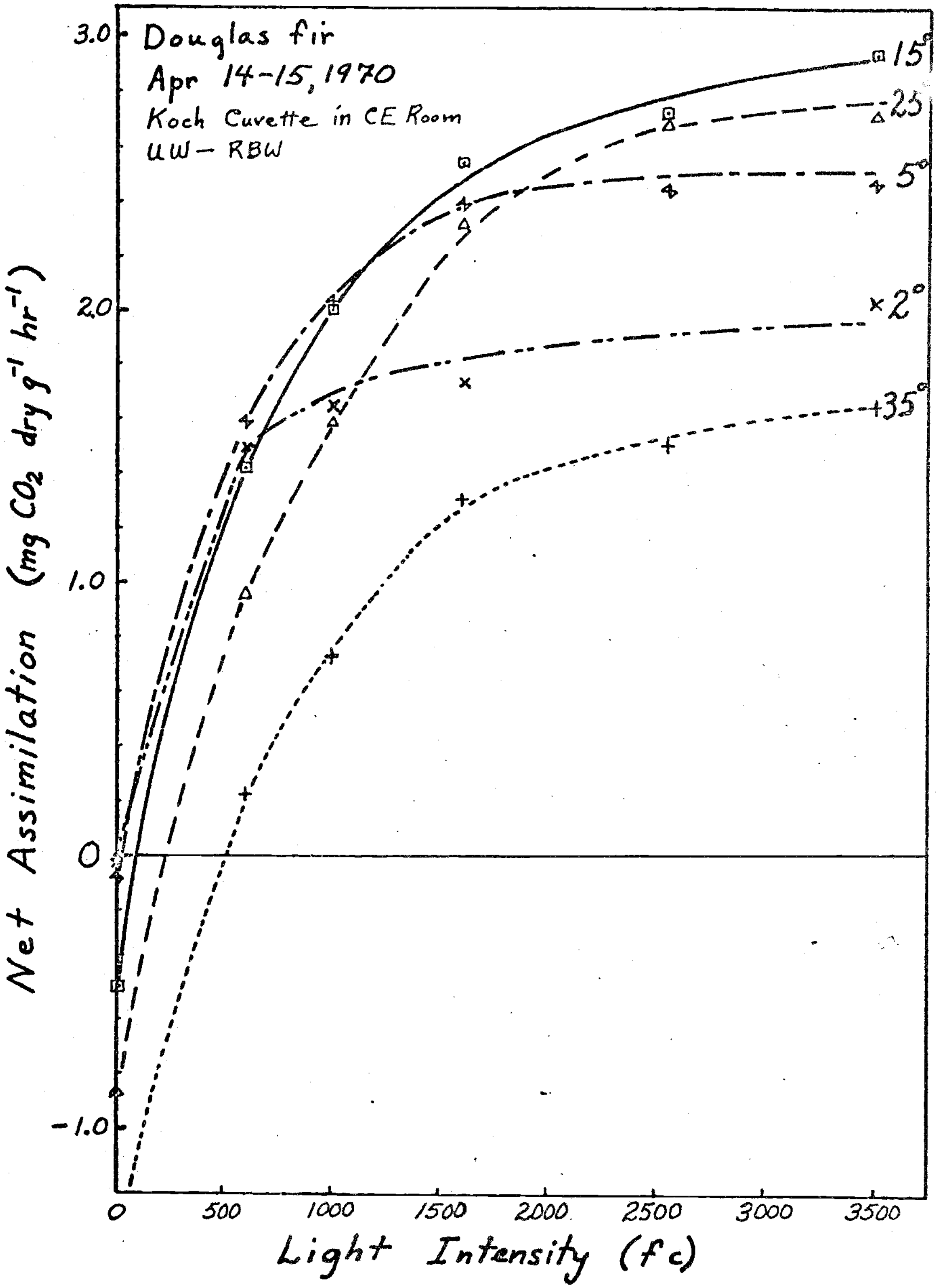
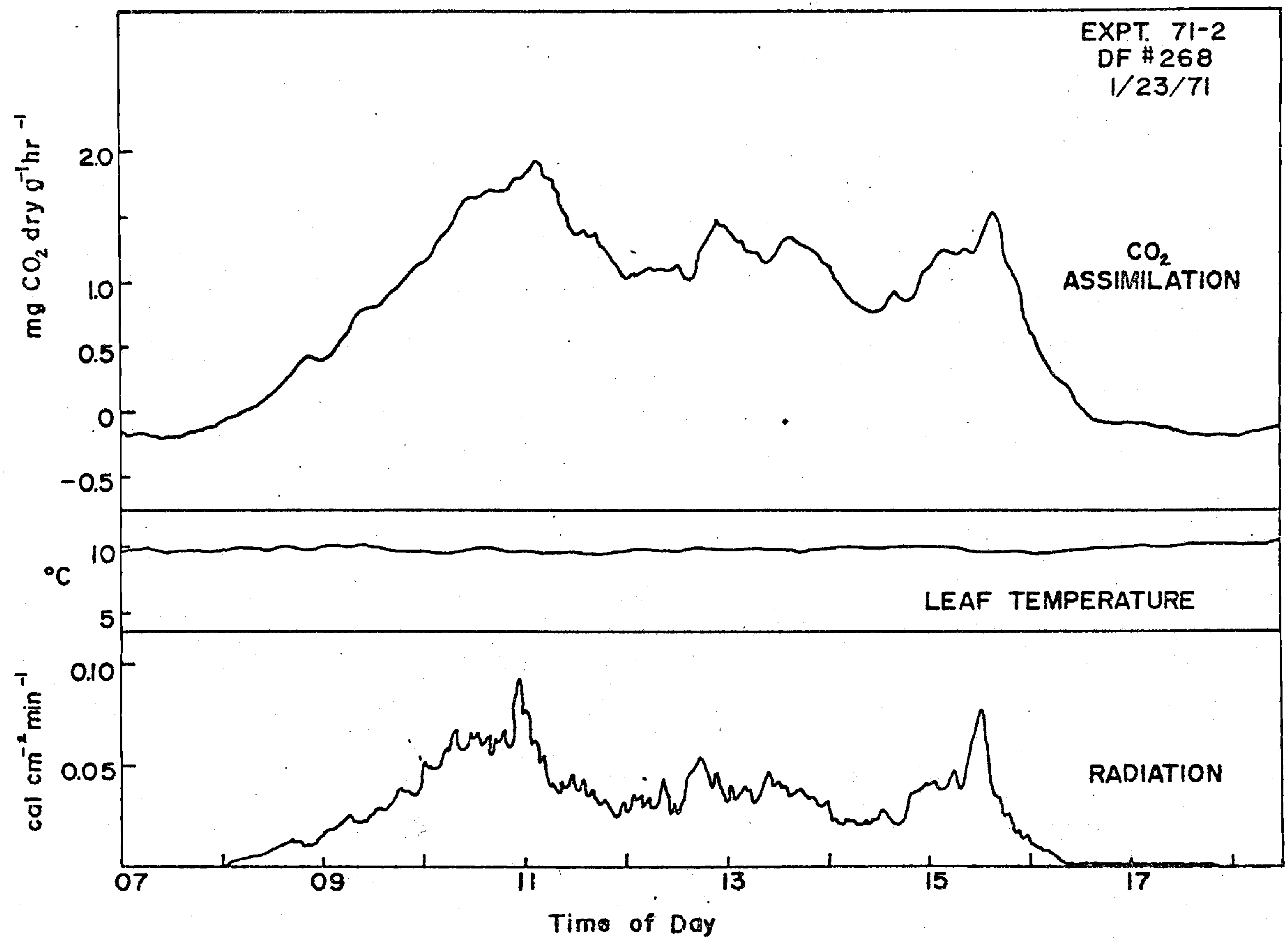

EXPT. 71-CR2
RUN 2 (BRANCH 2)
7/22-23/71
DF C
CO2 ASSIMILATION (mg CO2 dry g-1 hr-1)
Pstom
Ps
(atmospheres)
Rn (cal cm-2 min-1)
CUVETTE TEMPERATURE (°C)
TIME OF DAY
3
2
1
0
10
5
1.5
1.0
0.5
0.5
0.0
30
10
20
24
04
08
12
16

Figure 6


Douglas fir
Apr 14-15, 1970
Koch Cuvette in CE Room
UW - RBW
3.0
2.0
1.0
0
-1.0
15°
25°
5°
2°
35°
Net Assimilation (mg CO2 dry g-1 hr-1)
0
500
1000
1500
2000
2500
3000
3500
Light Intensity (fc)


EXPT. 71-2
DF #268
1/23/71
mg CO2 dry g-1 hr-1
2.0
1.0
0.5
0
-0.5
CO2
ASSIMILATION
°C
10
5
LEAF TEMPERATURE
cal cm-2 min-1
0.10
0.05
RADIATION
07
09
11
13
15
17
Time of Day

Figure

5. Increased P_{stom} (decreased stomatal aperature) was accompanied by decreased P_s (stress) and a further assimilation decrease.

5. P_s continued to rise and P_{stom} did not decrease.

CONTROLLED ENVIRONMENT AND GARDEN EXPERIMENTS ON THE INFLUENCES OF TEMPERATURE AND LIGHT INTENSITY

Richard B. Walker, David J. Salo, and James Nishitani

The influences of light intensity and temperature have received substantial attention in European conifers (Pisek and Winkler, 1958; Keller, 1965; Larcher, 1969) and some work has been done with Douglas-fir in the moderate temperature range (Krueger and Ferrell, 1965; Hodges and Scott, 1968; Brix, 1969). A scarcity of information concerning the performance of Douglas-fir at low temperatures, however, made clear the need for a study with emphasis in this range. This was of particular interest because of the frequency of lower temperatures during the cooler half of the year in the Pacific Northwest and at higher altitudes throughout the western states.

Experimental Methods and Equipment

The Koch-Siemens temperature-humidity controlled cuvette and back-up system was used for these experiments. The plants were Douglas-fir (*Pseudotsuga menziesii*) saplings 1 1/2 to 2 m tall and 8 to 10 years old grown from seed collected at Pack Demonstration Forest, La Grande, Washington. The young trees were in 8 to 20-liter cans in a sandy loam greenhouse soil. For the step-wise temperature experiments, the plants were held in a controlled environment room, with light varied by switching on appropriate numbers of the fluorescent and filament lamps in the overhead light bank. The soil was watered daily to draining using 1/10-strength Hoagland-Arnon solution 2 without micronutrient supplement, except that the plants outside received only natural rainfall, which was abundant during the season involved. For the controlled-environment experiments, the room was maintained at or near the cuvette temperature, except that the minimum room temperature was 8°C. Root temperatures were subject to the room temperature except in the special experiments involving root temperature, when the root cans were enclosed in isolated boxes with ice used to control the temperature to ± 1°C.

All measurements for a particular set of light intensity-temperature curves were made on a single branch. Any particular light or temperature regime was held long enough for a stable CO_2 differential to be recorded. This was usually 15 to 30 minutes. At the beginning and end of each run, a series of light intensities was run at 10°C to verify that the twig had remained at the same photosynthetic capacity during the experiment. This was always less than 10% of the original values. Depletion of carbon dioxide was prevented from exceeding 40 ppm by increasing the flow rate.

Results

Effects of temperature and light intensity. Special attention was paid to assimilation below 10°C, because of particular interest in performance at lower temperatures during the autumn, winter and spring. Figure 9 shows results of assimilation trials in this range of temperature. It was not feasible for us to operate the equipment below 0°C, but as the rate at 0°C was about 2/3 of that at 10°C, the needles must be capable of positive net assimilation at temperatures well below 0°C. In fact, at light intensities below 250 f-c, the rates at 0° and 2°C were at least double that at 10°C.

With other species there have been reports of changes in photosynthetic capacity during the year, usually involving higher capacities during the summer period. We measured at a range of temperatures during April, June, September, and November, but did not observe any sharp change over this period. Figures 7 and 10 show the patterns of the net assimilation during this period. Maximum net photosynthesis was reached at 10° to 15°C, and rates were nearly as high at 2° to 5°C, but fell markedly at 25° and 35°C because of high respiratory activity.

Effect of root temperature and water stress. As shown in Figure 11, net assimilation was essentially unaffected by root temperature in the range of 10° to 20°C, although at the highest leaf temperature tested (20°C), there was a possible downward trend. This downward trend was a little more evident at lower Scholander pressures (-15 bars) than at higher Scholander pressures (-5 bars). The lower Scholander pressures (P_s) were achieved by allowing soil moisture to deplete.

Effects of radiation intensity and temperature out of doors. These records were attained by using the Koch-Siemens cuvette on a 2 1/2 m tree in the garden outside the controlled environment area with the cuvette following the ambient temperature. The soil was always well-watered during this mid-winter period, and Scholander pressures taken in the early morning did not exceed 6 bars. Stomatal infiltration pressures were read frequently during the several weeks of measuring, and did not exceed 0.7 bar, indicating continually open stomata. Total radiation was measured using a Kipp and Zonen solarimeter.

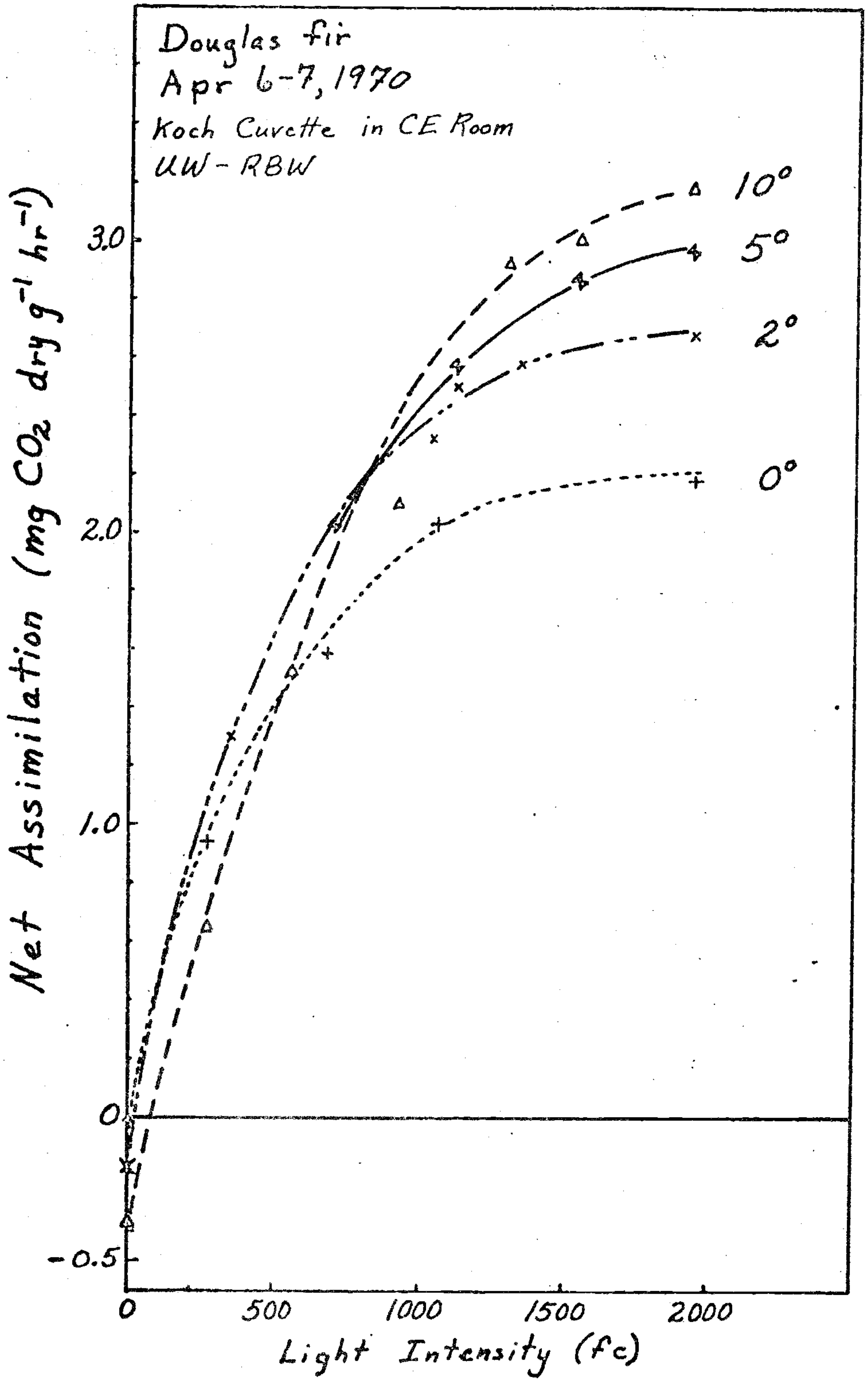
Figure 8 shows the results of a typical mid-winter day, with mid-day temperature about 10°C. Radiation was low throughout the day, and was followed very closely by the curve of net assimilation, indicating that light was limiting.

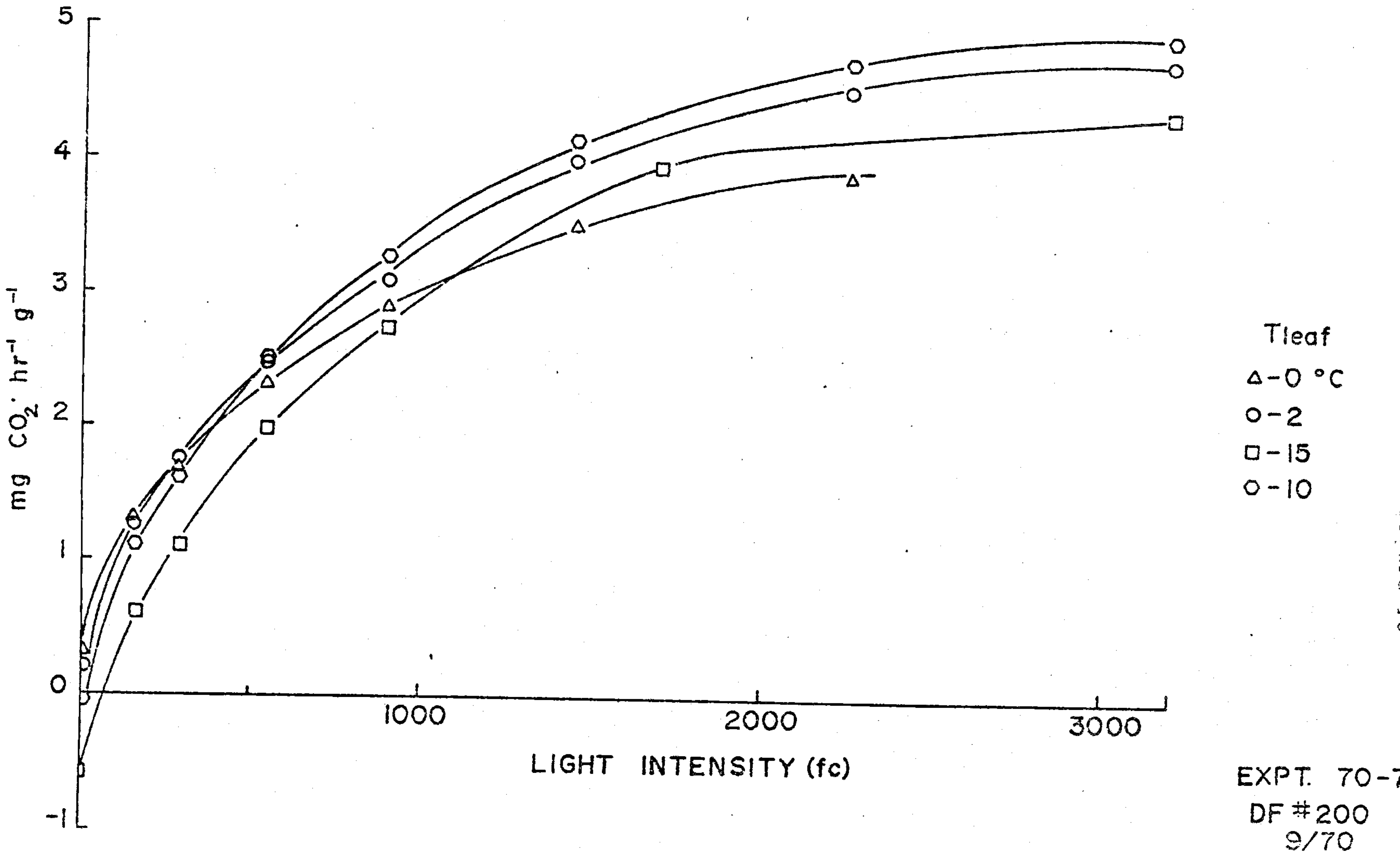
Figure 12 shows a January day with unusually high radiation, especially during midday. The curve of net assimilation followed the radiation curve in early morning and late afternoon, but was apparently temperature-limited during mid-day.

Summary

Experiments were conducted with 1 1/2 to 2 m tall Douglas-fir saplings, using the Koch-Siemens climate-controlled cuvette system. Principal results were:

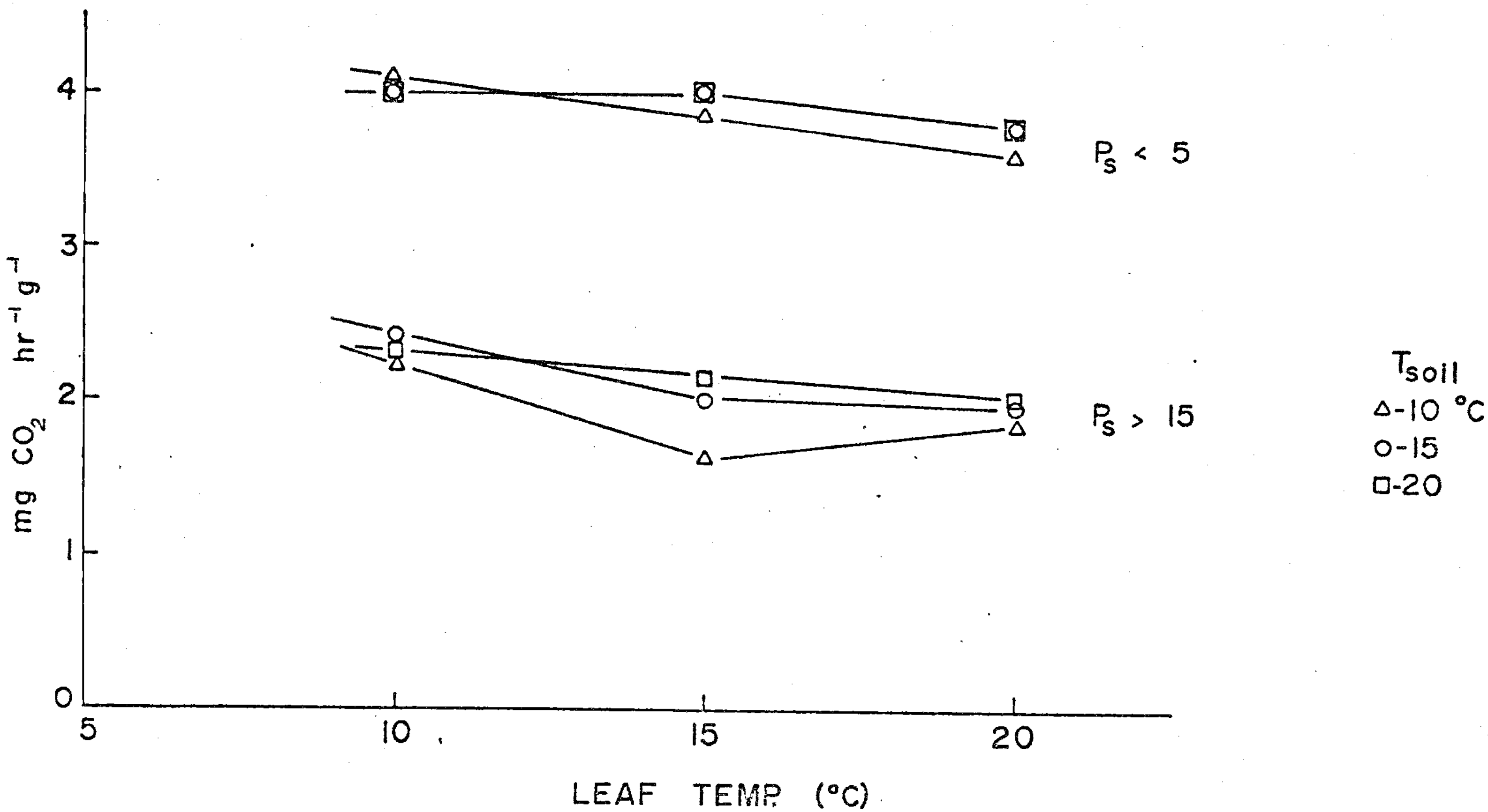
1) Curves of assimilation vs light intensity were developed. These showed that the optimal temperature for net photosynthesis was 10° to 15°C, with a marked decline above 15°C as expected. Net


Douglas fir
Apr 6-7, 1970
Koch Cuvette in CE Room
UW - RBW
10°
5°
2°
0°
Net Assimilation (mg CO_2 dry g^{-1} hr^{-1})
3.0
2.0
1.0
0
-0.5
0
500
1000
1500
2000
Light Intensity (fc)



5
4
3
2
1
0
-1
mg $CO_2 \cdot hr^{-1}\ g^{-1}$
1000
2000
3000
LIGHT INTENSITY (fc)
Tleaf
△-0 °C
○-2
□-15
○-10
EXPT. 70-7
DF #200
9/70

Figure 10



EXPT 70-8
DF #200
70

Figure 11

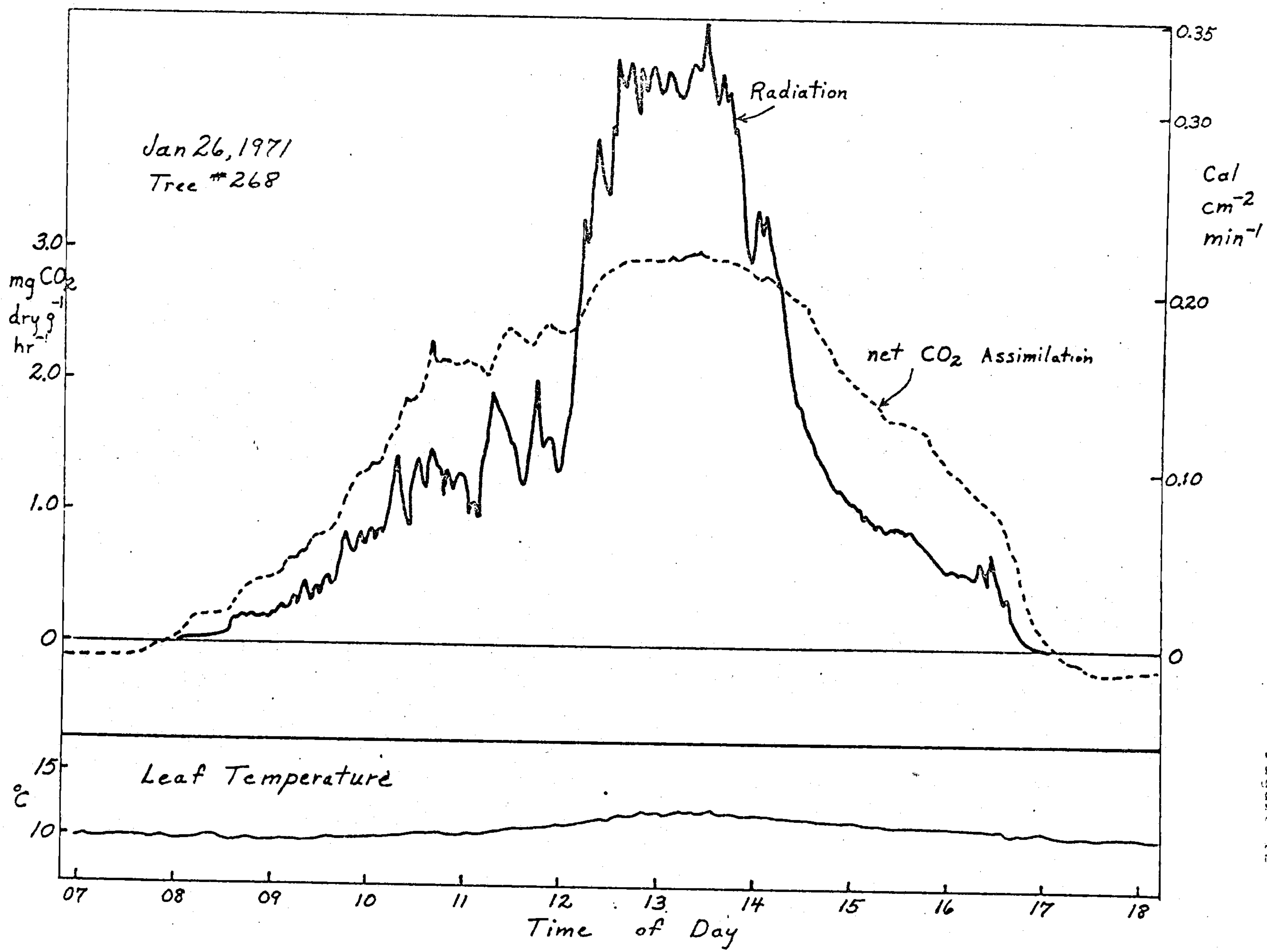

Jan 26, 1971
Tree #268
Radiation
net CO2 Assimilation
3.0
2.0
1.0
0
mg CO2 dry g-1 hr-1
0.35
0.30
0.20
0.10
0
Cal cm-2 min-1
Leaf Temperature
15
10
°C
07
08
09
10
11
12
13
14
15
16
17
18
Time of Day

Figure 12

assimilation at 0°C was about 2/3 that at 10°C at higher light intensities (1500-2000 f-c), but was at least twice as high as at 10°C at low light intensities (250 f-c).

2) Alteration of root temperature from 10° to 20°C had no measurable effect on net assimilation with leaf temperatures of 10°, 15°, or 20°C.

3) The net photosynthesis of a tree outside in the garden followed total radiation very closely on dull days in mid-winter. On the occasional day with sun during the middle of the day, light was no longer limiting the net assimilation, which was presumably temperature-limited during these period.

TENTATIVE FUTURE INVESTIGATIONS

Because of the limited data collected to date, it will be necessary to verify results reported above during the ensuing year. An attempt will be made to examine carefully the relationships that exist between assimilation, water stress, and environmental parameters such as temperature and light during the next twelve months to determine seasonal variations in effect of various factors on assimilation, as well as those occurring on a daily basis. This will supply essential data for the construction of mathematical models of assimilation.

Later, these studies need to be extended to different ages of Douglas-fir and to other important species, notably Western hemlock.