Tree Physiology 15, 175–179

A simple, battery-operated, temperature-controlled cuvette for respiration measurements

ROBERT M. HUBBARD,[1] MICHAEL G. RYAN[1] and DONAL L. LUKENS[2]

[1] *Rocky Mountain Experiment Station, 240 West Prospect Street, Fort Collins, CO 80526-2098, USA*

[2] *Department of Atmospheric Science, University of Wyoming, Laramie, WY 82071, USA*

Received May 24, 1994

Summary We designed a simple, portable, battery-operated, temperature-controlled cuvette to measure respiration of small samples of plant tissue in the field. The cuvette is built around a peltier cell and is controlled with a data logger. The cuvette maintained sample temperature within 0.5 °C over a temperature range of 5 to 45 °C and operated for 6–8 h from a 12 V 105 ampere-hour "deep-cycle" battery. Based on measurements with this cuvette, we found that, at 15 °C, CO_2 efflux from dark respiration of white pine (*Pinus strobus* L.) foliage was 40% greater during the day than at night.

Keywords: autotrophic respiration, temperature response.

Introduction

Respiration of living cells in foliage, woody tissue and roots can consume more than 60% of the carbon fixed in photosynthesis (Edwards et al. 1980), but information on respiration rates, and especially ecosystem-level estimates of fluxes, is sparse (Ryan et al. 1994). Because autotrophic respiration may control productivity and affect ecosystem responses to global change (Ryan 1991), studies on the physiology of autotrophic respiration should provide data to determine flux rates, to separate respiration into functional components of construction and maintenance, to determine response to environment, to scale measurements from chamber to canopy, and to determine whether acclimation to environment occurs. For many of these studies, temperature control of field measurements is necessary.

Because respiration is very sensitive to temperature and temperature often varies throughout a measuring period, measurements taken at ambient temperature must be adjusted to a common temperature to be comparable. Temperature control eliminates the need for adjustment and makes direct comparisons possible, facilitating comparisons within a canopy or across seasons. Additionally, temperature control can be used to generate temperature response curves during a single measuring period for use in modeling temperature response and in process-oriented models of carbon balance in forests (e.g., Forest-BGC (Running and Coughlan 1988), Hybrid (Friend et al. 1993) and Biomass (McMurtrie et al. 1990)). This method is preferable to generating response curves based on seasonal differences in temperature, where changes in phenology and growth are confounded with differences in temperature.

Temperature-controlled cuvettes have been used for several decades to examine gas exchange of plant tissue under controlled conditions (Schulze 1972, Bingham and Coyne 1977, Oechel and Lawrence 1979, Bingham et al. 1980, Kaul and Reisener 1980, Field et al. 1982, Atkinson et al. 1986, Küppers et al. 1987). However, these cuvettes are typically part of mobile laboratories or large fumigation systems, are large and difficult to build, and usually require line power for operation (Koch et al. 1971, Schulze 1972, Legge et al. 1978, Oechel and Lawrence 1979, Kaul and Reisener 1980, Atkinson et al. 1986, Küppers et al. 1987, van Hove et al. 1988). These characteristics make them unsuitable for use in remote locations. Although several lightweight cuvettes have been designed that use batteries as a power source, their componentry is inefficient and outdated compared with that now available (Salo et al. 1972, Bingham and Coyne 1977, Bingham et al. 1980).

In this paper, we describe in detail a simple, portable, battery-operated, temperature-controlled cuvette for use with small samples of foliage, fine roots, small stems and soils, and document its performance. The cuvette design was based on an earlier design by R.O. Teskey (University of Georgia) used to study foliage and fine root respiration by Cropper and Gholz (1991). The original design required line power, had a bulky commercial temperature controller, and used resistive heaters rather than a peltier cell to heat the cuvette.

Temperature-controlled cuvette

The temperature-controlled cuvette was built around a 12-V cold plate (TECA Inc., Chicago, IL) that incorporates a peltier cell to alter the temperature of attached components (Appendix 1). The temperature of the cuvette is controlled with a datalogger (Campbell 21X, Campbell Scientific, Logan, UT) and a series of solid-state and mechanical relays for switching power on and off and changing the polarity of the current for the peltier cell. (Source code for the datalogger control program is available from the senior author.) Power for the cuvette is supplied by a 12 V, 105 ampere-hour "deep-cycle" battery. The cuvette is capable of generating and maintaining temperatures from 2 to 45 °C at ambient temperatures of −10 to +35 °C. The system can run for 6 to 8 h on a fully charged battery.

Cuvette construction

The cuvette (Figure 1) is constructed of clear acrylic and is secured onto the cold plate with a neoprene gasket that provides an air-tight seal. A small heat sink attached to the cold plate promotes rapid heat transfer between the air inside the chamber and the peltier cell. A small 12-V fan mixes air within the chamber. The chamber is insulated with closed-cell neoprene foam. The top of the chamber is secured to the body with spring-loaded clamps. Plant material is secured within the chamber by placing it between the removable top and the body of the chamber, both of which are fitted with closed-cell neoprene foam to provide an air-tight seal. There is an inlet and an outlet port on either side of the chamber body to allow passage of the cuvette air through an infrared gas analyzer (Field et al. 1991).

The chamber in Figure 1 is only one of many possible designs that could be used with the cold plate system. However, the smaller the chamber, the faster the rate of temperature adjustment and the shorter the response time for CO_2 measurement. We have used the cuvette to provide stable temperatures for measuring autotrophic respiration of foliage and fine roots, and to measure the response of respiration to temperature. However, the cuvette could be adapted for photosynthesis measurements by modifying the chamber to allow the full spectrum of photosynthetically active radiation to be transmitted. Because heating of the chamber will occur during photosynthetic measurements, the control program may need to be modified to account for the increased heat load; increasing peltier cooling within the program is possible but would decrease operating time between battery charges.

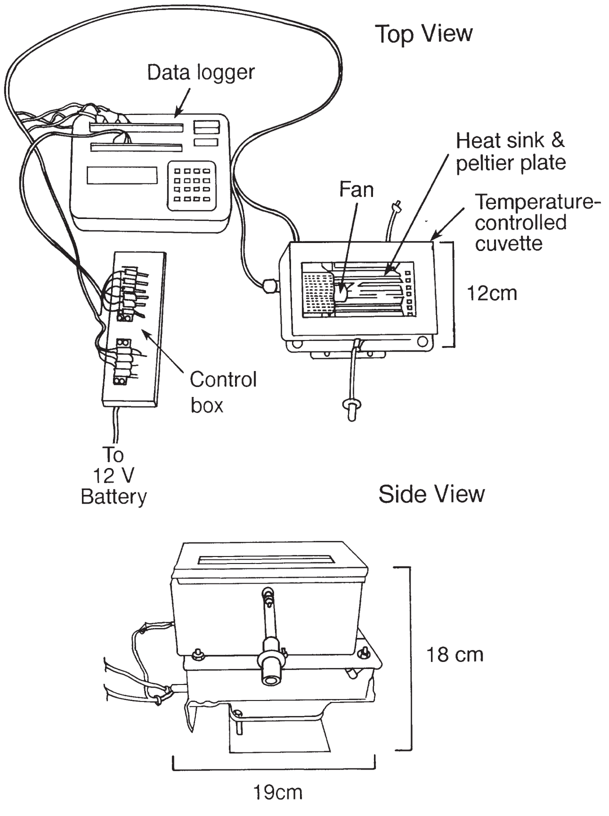

Figure 1. Temperature-controlled cuvette with component parts.

Temperature control

Temperature is monitored and controlled by a Campbell 21X datalogger that is programed to maintain sample temperatures within 0.5 °C of the set-point temperature. Tissue temperature is monitored with a 36-gauge fine wire copper-constantan thermocouple and controls a set of relays that turn the peltier cell on and off in addition to switching the direction of the current to the peltier cell.

The control circuit is shown in Figure 2. The datalogger monitors sample temperature (Thermocouple 1) and compares it to a user-selected set-point (Thermocouple 2 measures chamber air temperature to monitor the temperature gradient between foliage and chamber air). The datalogger control ports (CP1 and CP2) can supply about 20 mA of current at 5 V and switch solid-state Relays 1 and 3 (Figure 2, Appendix 1). Relay 1 provides current for mechanical Relay 2 (Figure 2, Appendix 1) which, in turn, switches the peltier cell current (about 6 A at 12 V DC) on and off. Relay 3 drives Relay 4, which switches polarity of the peltier cell current, thus heating or cooling the chamber.

There are four subroutines in the program and each is used to heat or cool depending on the difference between the set-point temperature and the actual sample temperature. "Fast" heating and cooling cycles are utilized when the difference between set-point and sample temperature is greater than 0.5 °C. "Slow" cycles are used when differences are less than 0.5 °C. As temperatures fall below or rise above set-point, the appropriate heating or cooling subroutine is initiated.

Because the peltier cell is relatively delicate, polarity is switched only after the peltier cell has been turned off. We also monitored peltier temperature (Thermocouple 3) to prevent overheating.

Testing and evaluation

To demonstrate the utility and performance of the temperature-controlled cuvette, we measured the response of CO_2 efflux from dark respiration of blue spruce (*Picea pungens* Engelm.) foliage on a detached branch (Figure 3). Before measurement, the branch end was cut underwater and the branch stored in the shade for 3 h. We measured CO_2 efflux at 5, 15, 25, 35 and 45 °C with an open-system infrared gas analyzer (LCA3, ADC Ltd., Hoddesdon, Herts, U.K.) with reference air (359 ppm) supplied from a pressurized gas cylinder. Temperature was altered every 40 min and the branch was shaded at all times.

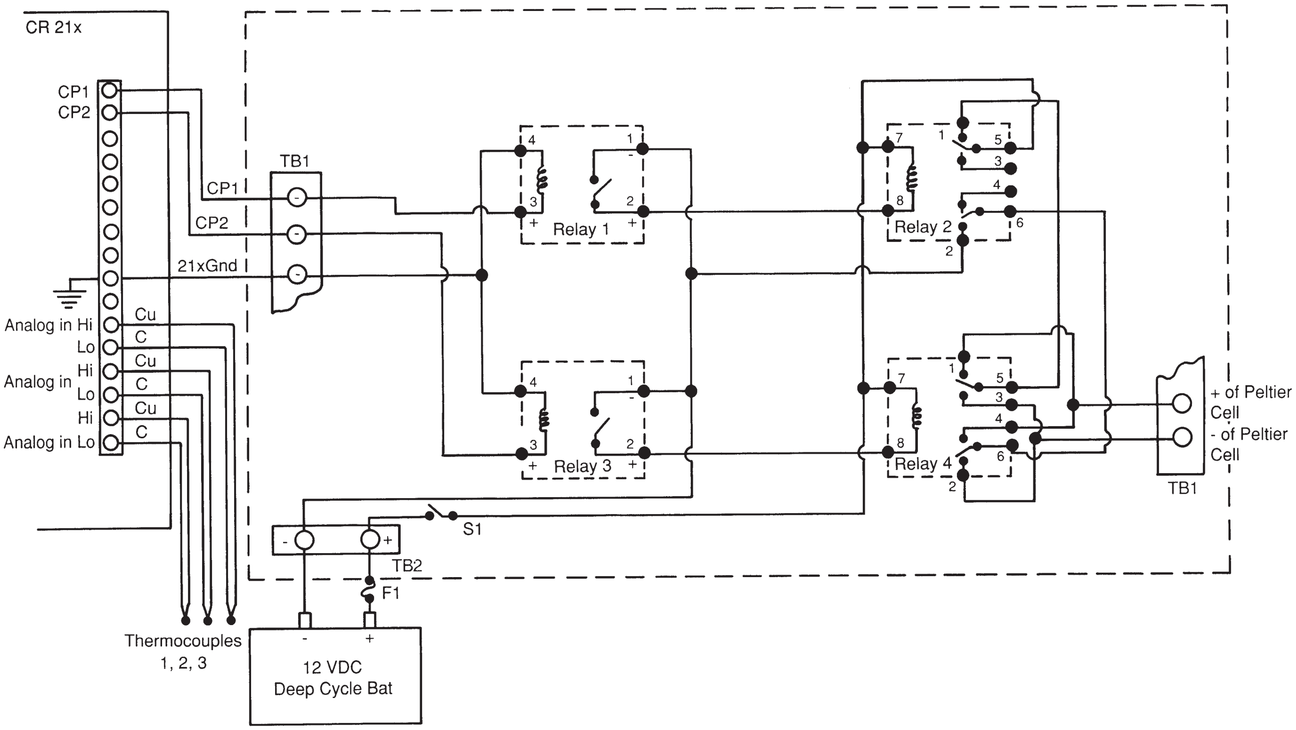

Figure 2. Circuit diagram for temperature-controlled cuvette. CR21X is Campbell datalogger, CP 1 and CP2 are datalogger control ports, Relays 1 and 3 are solid-state relays, Relays 2 and 4 are mechanical relays, S1 is an on/off switch, and F1 is an inline fuse. Pin numbers for the relays are specific to the relays we used (see Appendix 1).

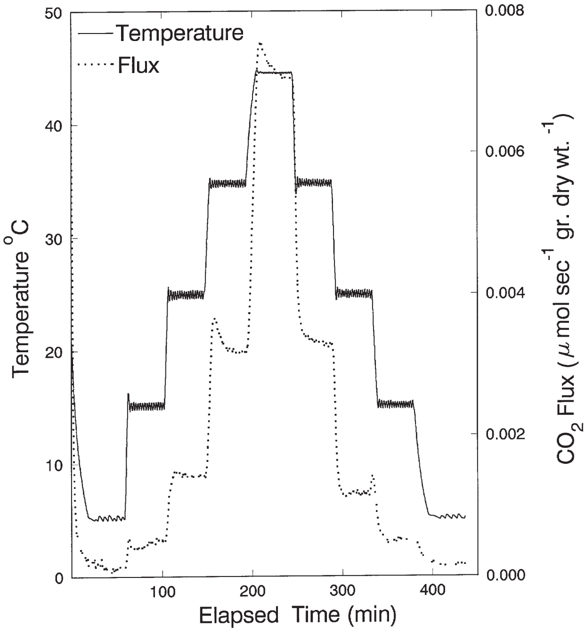

Figure 3. Temperature response of dark respiration in *Picea pungens* foliage. Each temperature was maintained for 40 min.

Based on the data in Figure 3, we averaged the middle 30-min flux values at each temperature and modeled the response of foliage respiration to temperature (T) by nonlinear regression analysis:

$$\hat{R} = \beta_0 e^{\beta_1 T}, \tag{1}$$

where $\hat{R}$ is the predicted respiration, and β_0 and β_1 are regression coefficients. Foliar Q_{10} was calculated as $Q_{10} = e^{(10\beta_1)}$ with a value of 2.3 for both increasing (5–45 °C) and decreasing (45–5 °C) temperature ranges.

Response time of the cuvette (time to reach new set-point) was relatively fast. Typically a 10 °C change in set-point temperature took approximately 6 min when temperatures were within 10 °C of outside air temperature and less than 12 min when temperatures differed by more than 10 °C from temperatures outside the cuvette. Carbon dioxide flux tracked temperature rapidly and approached a new equilibrium within 2–3 min (Figure 3).

The cuvette maintained temperatures within 0.5 °C of all set-point temperatures; however, temperatures achieved by the cuvette are slightly biased (Figure 4) at set-point temperatures of 5 and 45 °C, reflecting the large gradient between set-point temperatures and the temperatures outside the cuvette.

Application

To illustrate the utility of this cuvette, we compared CO_2 efflux from the same foliage of three shoots of eastern white pine (*Pinus strobus* L.) at 15 °C at midafternoon, midnight, before dawn and again at midafternoon. Before measure-

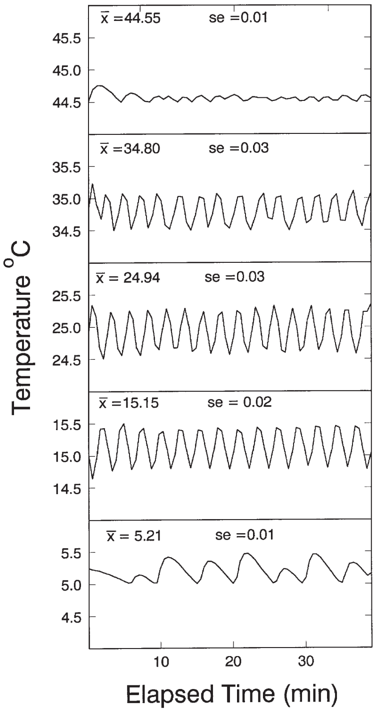

Figure 4. Foliage temperature at cuvette set-points of 5, 15, 25, 35 and 45 °C.

ments, the shoots were covered with breathable cloth bags for 1 h and held in the shade. The foliage was then removed from the bags under shade and placed in the darkened cuvette. Foliage was not covered with bags before measurement at night.

Carbon dioxide efflux was measured with an open-system infrared gas analyzer (LCA 3, ADC Inc., Hoddesdon, Herts, U.K.) with an air flow of 137 mmol s^{-1}. Reference air was drawn through a 20-l ballast, and data were recorded after CO_2 efflux and temperature were stable for 15 min. For the three shoots, dark respiration rates at 15 °C were 40% higher when measured during the day after 1 h of darkness than at night (Figure 5).

We found similar differences between day and night dark respiration rates measured at 15 °C for Florida slash pine (*Pinus elliottii* var. *elliottii* Engelm.), Wisconsin red pine (*Pinus resinosa* Ait.), Montana ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) and Oregon western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Ryan and Hubbard, unpublished data). Together, these data suggest that measurements of foliar dark respiration taken after 1 h of shade during the day may significantly overestimate dark respiration at night.

We conclude that the temperature-controlled cuvette allows a researcher to hold temperature effects constant when comparing respiration within a canopy or across seasons, and to generate temperature response curves for respiration of fine roots and foliage. We have found the cuvette a useful and versatile instrument for collecting a wide variety of ecophysiological data.

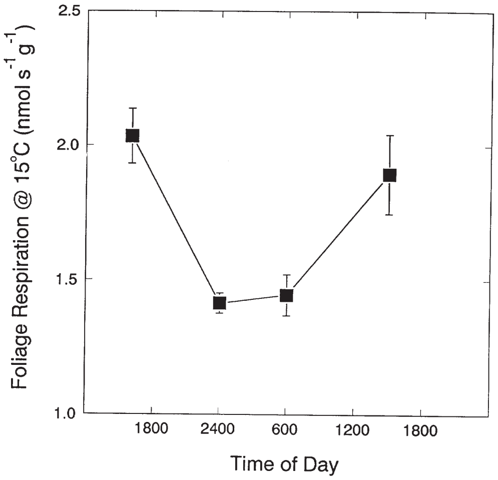

Figure 5. Efflux of CO_2 from three white pine shoots at 15 °C on December 6 and 7, 1991. Air temperatures for the four measurement periods were 10 °C at 1600 h, 1 °C at 2400 h, –1 °C at 0600 h, and 14 °C at 1500 h.

References

Atkinson, C.J., W.E. Winner and H.A. Mooney. 1986. A field portable gas-exchange system for measuring carbon dioxide and water vapor exchange rates of leaves during fumigation with SO_2. Plant Cell Environ. 9:711–719.

Bingham, G.E. and P.I. Coyne. 1977. A portable, temperature-controlled, steady-state porometer for field measurements of transpiration and photosynthesis. Photosynthetica 11:148–160.

Bingham, G.E., P.I. Coyne, R.B. Kennedy and W.L. Jackson. 1980. Design and fabrication of a portable minicuvette system for measuring leaf photosynthesis and stomatal conductance under controlled conditions. Lawrence Livermore National Lab., Livermore, CA, Report No. UCRL-52895, 64 p.

Cropper, W.P. and H.L. Gholz. 1991. *In situ* needle and fine root respiration in mature slash pine (*Pinus elliottii*) trees. Can. J. For. Res. 21:1589–1595.

Edwards, N.T., H.H. Shugart Jr., S.B. McLaughlin, W.F. Harris and D.E. Reichle. 1980. Carbon metabolism in terrestrial ecosystems. *In* Dynamic Properties of Forest Ecosystems, International Biological Program 23. Ed. D.E. Reichle. Cambridge University Press, Cambridge, pp 499–536.

Field, C.B., J.A. Berry and H.A. Mooney. 1982. A portable system for measuring carbon dioxide and water vapor exchange of leaves. Plant Cell Environ. 5:179–186.

Field, C.B., J.T. Ball and J.A. Berry. 1991. Photosynthesis: principles and field techniques. *In* Plant Physiological Ecology. Eds. R.W. Pearcy, J. Ehleringer, H.A. Mooney and P.W. Rundel. Chapman and Hall, New York, pp 210–251.

Friend, A.D., H.H. Shugart and S.W. Running. 1993. A physiology-based model of forest dynamics. Ecology 74:792–797.

Kaul, R. and H.J. Reisener 1980. A method with integrated temperature control to measure potential net photosynthesis in green plant tissues at high quantum flux densities. Z. Pflanzenphysiol. Bd. 96S:445–452.

Koch, W., O.L. Lange and E.-D. Shulze. 1971. Ecophysiological investigations on wild and cultivated plants in the Negev Desert. I. Methods: a mobile laboratory for measuring carbon dioxide and water vapor exchange. Oecologia 8:296–309.

Küppers, M., A.G. Swan, D. Tompkins, W.C.L. Gabriel and B.I.L. Küppers. 1987. A field portable system for the measurement of gas exchange of leaves under natural and controlled conditions: examples with field-grow *Eucalyptus pauciflora* Sieb. ex Spreng. ssp. *pauciflora*, *E. behriana* F. Muell. and *Pinus radiata* D. Don. Plant Cell Environ. 10:425–435.

Legge, A.H., D.J. Savage and R.B. Walker. 1978. Special techniques: a portable gas-exchange leaf chamber. *In* Methodology for the Assessment of Air Pollution Effects on Vegetation: A Handbook. Eds. W.W. Heck, S.V. Krupa and S.N. Linzon. Air Pollut. Control Assoc., Brussels, pp 12–24.

McMurtrie, R.E., D.A. Rook and F.M. Kelliher. 1990. Modeling the yield on *Pinus radiata* on a site limited by water and nitrogen. For. Ecol. Manage. 39:381–413.

Oechel, W.C. and W.T. Lawrence. 1979. Energy utilization and carbon metabolism in Mediterranean scrub vegetation of Chile and California. Oecologia 39:321–335.

Running, S.W. and J.C. Coughlan. 1988. A general model of forest ecosystem processes for regional applications. I. Hydrologic balance, canopy gas exchange and primary production processes. Ecol. Model. 42:125–154.

Ryan, M.G. 1991. The effect of climate change on plant respiration. Ecol. Appl. 1:157–167.

Ryan, M.G., S. Linder, J.M. Vose and R.M. Hubbard. 1994. Dark respiration in pines. *In* Pine Ecosystems. Eds. H.L. Gholz, S. Linder and R.E. McMurtrie. Ecological Bulletins 43, Uppsala, pp 50–63.

Salo, D.J., A. Ringo, J.H. Nishitani and R.B. Walter. 1972. Development and testing of an inexpensive thermoelectrically cooled cuvette. *In* Proc. Research on Coniferous Forest Ecosystems: A Symposium. Eds. J.F. Franklin, L.J. Dempster and R.H. Waring. USDA Forest Service, Portland, OR, pp 273–277.

Schulze, E.-D. 1972. A new type of climatized gas exchange chamber for net photosynthesis and transpiration measurements in the field. Oecologia 10:243–251.

van Hove, L.W.A., W.J.M. Tonk, G.A. Pieters, E.H. Adema and W.J. Vredenberg. 1988. A leaf chamber for measuring the uptake of pollutant gases at low concentrations by leaves, transpiration and carbon dioxide assimilation. Atmos. Environ. 22:2515–2523.

Appendix 1. Parts list

Item and model number	Quantity	Company
Solid-state cold plate AHP-150	1	TECA Chicago, IL
Campbell datalogger tific (21X type)	1	Campbell Scien- Logan, UT
Solid-state relay D1D2 (3.5–32 V switching up to 12 A, 100 V DC output)	2	Crydom Corp.
Mechanical relay #2342 DPDT, 10 A coil, 12 V DC	2	Phillips Corp. Williams Port, PA
12 V Fan motor and blade 2522 846-11.112-000	1	Maxon Motors Burlingame, CA
36-gauge Copper-constantan thermocouple with mini-connectors	7 m	Omega Corp. Stamford, CT
16-gauge 4-conductor Power cord	3 m	