QUALITY ASSURANCE METHODS MANUAL

FOR

EXPOSURE SYSTEMS AND PHYSIOLOGICAL MEASUREMENTS

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PREFACE

The following Forest Response Program (FRP) Methods Manual for Exposure Systems and Physiological Measurements was developed in response to the Environmental Protection Agency's (EPA) requirements for Quality Assurance (QA) in environmental research. The Exposure Systems workshop which provided the basis for this document was held on March 11-12, 1986 at Raleigh, NC. The workshop was attended by 24 individuals consisting of Principal Investigators and Program Administrators (see Appendix A). The Exposure Systems workshop is one of four workshops conducted by the EPA to develop Quality Assurance Methods Manuals for the Forest Response Program. The workshops and ensuing reviews of the Methods Manuals were designed to allow scientists input in the selection of standard research methods and the development of QA procedures for these methods. Drs. Lance Evans and Phil Dougherty provided workshop leadership and compiled this methods manual from the comments and concerns voiced at the workshop, and other sources.

The protocols and Standard Operating Procedures (SOPs) presented in these methods manuals represent a consensus on the "best" technique for operating a specified system of equipment or measuring a specified variable given the various objectives of the Forest Response Program. "Best" is defined for this manual according to the following criteria:

1) the quality or soundness of the protocol or SOP;

2) the availability of staff and facilities to adhere to the protocol or SOP; and

3) the feasibility of assuring data quality through tests for accuracy, precision, and consistency among sites.

The protocols and SOPs written in this first edition of the manuals are general in nature and encompass most existing research plans. As the program progresses, the protocols and procedures will be revised, and the manuals will be expanded to include additional protocols and SOPs.

The purpose of the manual is (1) to provide standardization of research methods and design to allow for the synthesis and integration of results for assessment purposes; (2) to provide standard quality assurance/quality control techniques within standardized protocols and SOPs to allow for the assessment and documentation of data quality; and (3) to prevent duplication of documentation efforts among investigators using common techniques. Appropriate standardization of research methods which contribute data to a centralized data base is critical to the synthesis/integration/assessment effort for the Forest Response Program. Without comparability among sites, an overall assessment of research results would be impossible. The National Forest Response Program Research Plan provides details on the integration and assessment effort.

Standard quality assurance activities, providing guidelines for the minimum amount of activity required to participate in the integrated research program, ensure the ability of investigators and Program

Administrators to assess data quality as data are produced. This knowledge directly influences the level of confidence for assessment decisions, which is determined from the quality of the individual parts. Other aspects of the quality assurance program (research plan preparation, auditing, and sample exchanges between sites) contribute to the process to ensure that data are of known and sufficient quality to meet the program's objectives.

Finally, these methods manuals will serve the investigators in simply reducing the amount of documentation required for the assessment of data quality. Many of the techniques employed by investigators examining similar hypotheses are nearly identical. The manuals identify and document these similarities and can be referenced by the investigators in their research plans. Much of the information required by the QA program is included in these manuals; however, instances occur when generalities should be clarified in individual plans. For example, "sufficient training to operate the required equipment" as might be found in a manual must be specified for projects and facilities in the individual research plan since large differences may exist between sites and projects.

All investigators with research projects funded through the Forest Response Program Research Cooperatives will be required to adhere to the protocols and the SOPs as described in these manuals. Where necessary, investigators can deviate from the manual, by providing (1) a justification for the deviation; (2) a full explanation of the alternative protocol or procedure with QA activities clearly described; and (3) an assessment of the impact of the deviation on data quality, by comparing the alternative protocol or procedure with the original. This will create an additional documentation burden for the investigator and an assessment burden for administrators, and therefore, should be used only when absolutely necessary.

The Forest Response Program Quality Assurance staff would like to thank the workshop leaders, Dr. Evans and Dr. Dougherty, and the participants for their technical efforts in developing and refining the material in this manual, and the Acid Deposition Program staff for organizing and supporting the effort. We appreciate the cooperation and patience of all the investigators during the slow process of developing the QA program, including this manual. We look forward to the ensuing years of quality research and an integration and assessment effort worthy of that quality.

NOTE: The use of brand names in this manual is not an endorsement of a particular product but a guide to the type of equipment required to perform a particular measurement.

1. INTRODUCTION

The recently established Forest Research Cooperatives are charged with the task of understanding the nature of forest declines, if forest declines are occurring, as well as making an economic assessment of effects at some date in the future. This is an ambitious task and will only be accomplished if effective and efficient planning occurs before experimentation begins.

The purpose of an assessment is to present the best answers or predictions in response to policy questions with a clear discussion of all uncertainties (Moskowitz et al., 1985). For any assessment activity, only limited data will be available and certain inferences will be made from available data bases to predicted regional or national impacts. Of course, any prediction carries uncertainties. Most accurate predictions can be made when the magnitude of each uncertainty can be characterized or, at least, be placed within certain limits.

1.1. EXPOSURE SYSTEMS

This document is constructed with the protocols as outlined in the recent International Workshop on Standardization of Pollutant Exposure Systems and Protocols held in Corvallis, OR, in January 1986, as a foundation (Table 1) (Hogsett et al., 1986). Participants at the workshop recognized that the "ideal" system for multi-year growth studies with both seasonal and year-round exposure to different pollutant types would be structureless, i.e., not introducing artifacts of growth with hardware and shelters. Participants recognized that experimentation may involve certain tradeoffs. One tradeoff is the need to exclude ambient pollutants (wet and dry deposition), and a means to accomplish this is a chamber or enclosure. The automated exclusion systems, in use at some acid-rain deposition sites, represents an attempt to minimize the artifacts except during the ambient event (i.e., rain event). The workshop participants recognized that, since researchers lacked an "ideal" or structureless exposure system that was well-characterized for tree growth and pollutant delivery, there was value in employing some more adequately characterized exposure systems. Thus, this allows the opportunity of cross-comparability between research sites with some systems, but avoids the undesirable introduction of consistent bias into the programs. The workshop recognized the utility of a variety of exposure systems for multi-year growth studies, including both chambered and non-chambered type systems.

Some experimentation may need to take place under conditions in which mechanisms of response, initial stages of screening sensitive plant species and/or genetic variants, or other considerations such as accessibility to remote areas or characteristics of the environment may make certain exposure systems inappropriate. Under such conditions it may be necessary to conduct wet deposition studies under a permanent exclusion cover or in a controlled environment facility. In a likewise manner, dry deposition studies may need to be conducted in controlled environment

conditions. It is acknowledged that none of the exposure systems have been adequately tested for performance with forest trees. Regardless of system(s) used, tree growth and physiology should be accurately characterized so that pollutant effects can be separated from exposure system effects. Nevertheless, it is recognized that the more the experimental environment differs from the natural environment, the more problematic inferences become when experimental results are used for regional or national assessments.

Table 1. Recommended exposure systems for combinations of dry and wet deposition. (The order does not imply prioritization.) Table is printed from Table VI-6 of the report of the International Workshop on Standardization of Pollutant Exposure Systems and Protocols, Corvallis, OR, January 1986. (Hogsett et al., 1986)

Multi-Year Growth/Physiology Studies		
Wet Deposition	--	Automated or manual exclusion/scheduled addition
Dry Deposition	--	Chamberless exposure system
Wet Deposition	--	Automated or manual exclusion/scheduled addition
Dry Deposition	--	Open-top chamber technology
Wet Deposition	--	Permanent exclusion/scheduled addition
Dry Deposition	--	Open-top chamber technology
Short-Term Growth/Physiology Studies		
Same as multi-year plus indoor chambers		

1.2. PHYSIOLOGICAL MEASUREMENTS

The purpose of the physiological measurement section is to define acceptable standards for measuring the key physiological processes that are of interest to the Forest Response Program. The measurements addressed in this manual are those made at the whole plant or organ level and not those made at the subcellular or biochemical level.

Exact methods, numbers of plants to be sampled, and frequency of sampling cannot be specified for all studies because they will be a function of study objectives. The accuracy and precision of each measurement has been specified where possible for each of the physiological measurements considered in this document. This will require that equipment with suitable resolution and stability be used. Otherwise, specific equipment recommendation has been avoided.

In this section of the manual it has been proposed that standard units and recording formats be accepted for each physiological process measured. Adherence to these guidelines will make data management and data analysis easier.

1.3. REFERENCES

Hogsett, W. E., D. P. Ormrod, D. Olszyk, G. E. Taylor, Jr., and D. T. Tingey. 1986. Air Pollution Exposure Systems and Experimental Protocols: A Review and Evaluation of Performance. (In preparation)

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2. PROTOCOL NARRATIVES FOR EXPOSURE SYSTEMS

2.1. WET DEPOSITION - AUTOMATIC RAINFALL EXCLUSION WITH REGULAR ADDITIONS

Automatic rainfall exclusion systems equipped to apply simulated precipitation on a regular schedule were recommended for wet deposition studies by the International Workshop on Standardization of Pollutant Exposure Systems and Protocols held at Corvallis, Oregon, during January 1986 (Hogsett et al., 1986). The concept behind the use of such facilities centers around the need to exclude all ambient wet deposition and apply deposition of known chemistry, quantity, etc. as well as the need to change the microclimate of the environment only minimally so that the experimental environment most nearly mimics the natural environment. These systems have been constructed at several sites in North America based upon the facility at Brookhaven National Laboratory (Lewin and Evans, 1985). These facilities do not exclude dry deposition.

2.1.1. SYSTEM CHARACTERISTICS

- These facilities should be made of construction materials such that no structural damage should occur at wind speeds of up to 23 m/s at low elevations.
- At high elevations (above 1000 m) the structures should be able to withstand wind speeds of up to 41 m/sec.
- The exclusion system should exclude ambient precipitation within three minutes after sensors experience the first droplet.
- The shelter should be moved off the experimental area within five minutes after precipitation stops.
- A real-time recorder system must monitor when the shelter is over the experimental area.
- All records of time are to be reported in standard military time.
- No surface water should enter the exclusion area.
- The simulated rain distribution system should be a permanent part of the shelters and should not be in the experimental area unless the shelter is over the area.
- The covering of the shelters should not transmit less than 75% of ambient light - 90% is recommended level, with transmission to 75% of ambient in the PAR spectrum region. If the light intensity decreases below this level, the cover should be cleaned or changed.
- The temperature of the treatment area should not differ by more than 3°C from ambient.

- The relative humidity of the air of the treatment area should not differ by more than 10% from ambient.

- All instruments to determine and record temperature and relative humidity should be calibrated monthly during experimentation.

- Climatological data of ambient conditions should be recorded continuously. Wind speed within the shelter during simulated precipitation events must be below 1 m/sec so that the pattern is not significantly affected.

2.2. DRY DEPOSITION - OPEN-AIR RELEASE (CHAMBERLESS) SYSTEMS

Chamberless exposure systems were recommended by the International Workshop (Hogsett et al.,) for dry deposition studies attempting to mimic natural conditions. The chief advantage of open air release (structureless exposure) systems is that they dispense gaseous air pollutants with a minimum of perturbations of the natural environment and, as a result, experimental results from such experiments are most applicable for assessment purposes. One limitation of these systems is the lack of an imposed "no pollution" control unless the experimental field is located in such an environment or a gas without pollutants is administered. Many systems have been developed (de Cormis et al., 1975; Lee and Lewis, 1978; Heitschmidt et al., 1980; Muller et al., 1979; Miller et al., 1980; Greenwood et al., 1982; McLeod et al., 1985; Mooi and van der Zalm, 1985a, 1985b). The performance records of the systems described by Greenwood et al. (1982), McLeod et al. (1985), and Mooi and van der Zalm (1985a, 1985b) are probably the most complete and were used as guides for this report.

The configuration of pipes used by Mooi and van der Zalm (1985a, 1985b) is a new development. They used 16 pipe segments in a circular array in such a way that each pipe could be operated independently. In this manner, each of the 16 pipe segments would supply test gases to only 22 (360/16) degrees within the circle's area. Only pipes up-wind of the experimental area are activated at any one time. The concentration of SO_2 in the experimental area is maintained by computer. Results of tests during the latter portion of 1984 show that the average concentration in the center of the experimental field could be maintained at 200 $\mu g/m^3$ SO_2. The deviation of this average was about 15% (average of 10 consecutive measurements within a period of 16 minutes). Higher peaks were extremely rare. Distribution of SO_2 gas over the whole experimental field (diameter 10 m) had a deviation of about 20%. This level of control should be attempted in all experiments.

To operate this system, the following environmental data must be monitored continuously within the experimental area:

- wind speed in m/s
- wind direction
- temperature in degrees Celsius

- pollutant concentration at each sample point (at least three sampled sites at two heights per test plot)
- background concentrations of pollutant.

The QA documentation needs a description of how the automated system operates. In case of perceived alarms in the systems, the following problems will call for particular actions. The alarms and actions are:

1. If pollutant concentration is too high (20% above set point), injection will be reduced markedly.

2. The automatic system should be checked periodically.

3. All instruments should be calibrated according to schedules that follow.

4. An instrument should be dedicated to surveillance of one system on a constant basis.

5. All gas distribution lines should be monitored frequently to assure accurate flow rates.

6. QA checks should be made by adding pollutants and monitoring how the automated system responds.

The characteristics of performance for O_3, SO_2, and NO_x analyzers are shown in Table 2. For open field exposure conditions, a low detectable limit, high degree of specificity, high stability, and rapid response time are very important. Such characteristics are less important when chamber conditions are used, because the pollutant concentrations can be controlled more accurately. For open air situations, the following conditions should be monitored during the experimental period.

1. Atmospheric Chemistry

   (A) Gaseous Pollutants

      (1) Distribution Patterns
         (i) Horizontal -- continuous monitoring at many points in a grid over plot (minimum of three points)
         (ii) Vertical -- systematic monitoring at two heights in each plot (minimum of two points)

      (2) Temporal Patterns -- continuous at many points

   (B) Non-Pollutant Chemicals

      (1) Water Vapor (Humidity) -- continuous at one point in ambient air

      (2) CO_2 -- not applicable

Table 2. Performance characteristics of many gaseous pollutant analyzers.

TYPICAL OZONE/OXIDANT ANALYZERS

Parameter	Analyzer: Colorimetric	Gas-Phase Chemiluminescent	Gas-Solid Chemiluminescent	UV Photometric	Electrochemical
Lower Detectable Limit	10-20 ppb	< 1-10 ppb	< 1-10 ppb	< 1-10 ppb	10-20 ppm
Specificity[a]	low	high	high	high	low
Stability[b]	low	high	high	high	low
Response Time	< 5 min	< 1 min	< 1 min	< 1 min	< 5 min
Working Range	up to 10 ppm	up to 2 ppm	up to 2 ppm	up to 10 ppm	up to 10 ppm
Cost (dollars)	3-6 K	3-6 K	> 6 K	3-6 K	1-3 K

TYPICAL SO_2 ANALYZERS

Parameter	Analyzer: Conductimetric	Voltametric	Amperometric	Colorimetric	Flame Photometric	2nd Derivative UV Spectrometric	Pulsed UV Fluorescent
Lower Detectable Limit	10-20 ppb	10-20 ppb	10-20 ppb	10-20 ppb	< 1-10 ppb	10-20 ppb	10-20 ppb
Specificity[a]	moderate	moderate	moderate	high	moderate	high	moderate
Stability[b]	low	low	high	low	high	high	high
Response Time	< 5 min	< 5 min	< 5 min	< 5 min	< 1 min	< 5 min	< 5 min
Working Range	up to 10 ppm	up to 10 ppm	up to 2 ppm	up to 4 ppm	up to 10 ppm	up to 2 ppm	up to 5 ppm
Cost (dollars)	1-3 K	1-3 K	3-6 K	3-6 K	> 6 K	< 6 K	> 6 K

TYPICAL NO_x ANALYZERS

Parameter	Analyzer: Voltametric	Amperometric	Colorimetric	Chemiluminescent
Lower Detectable Limits	10-20 ppb	10-20 ppb	10-20 ppb	< 1-10 ppb
Specificity[a]	low	low	high	low
Stability[b]	low	low	low	low
Response Time	< 5 min	< 5 min	> 5 min	< 1 min
Working Range	up to 10 ppm	up to 12 ppm	up to 2 ppm	up to 10 ppm
Cost (dollars)	1-3 K	3-6 K	3-6 K	< 6 K

[a]Specificity: high -- < 10% error from species commonly encountered in ambient air; moderate -- scrubber required to eliminate interferences; low -- scrubber may not eliminate interferences under all conditions, and/or data corrections required based on concurrent measurements.

[b]Stability: high -- meet EPA Reference and Equivalent Method specifications; moderate -- may be operated without significant drift for 1-2 days; low -- requires daily zero/span adjustment.

Source: Burmann and Rehme (1978).

2. Physical Properties of the Experimental Area

A meteorological station should be established to monitor the ambient environment. This station should be constructed and operated according to US EPA guidelines.

(A) Irradiance (Quantum Level at 400-700 nm)

(1) Distribution -- not applicable

(2) Temporal Patterns -- Continuous monitoring at one point in ambient air

(B) Air Temperature -- Continuous at one point in ambient air

(C) Air Movement -- Continuous wind speed measurements are necessary as stated above

(D) Soil Temperature -- Periodically at one point in ambient plot area

2.3. WET DEPOSITION - AUTOMATIC RAINFALL EXCLUSION WITH AUTOMATIC ADDITIONS

The International Workshop (Hogsett et al., 1986) recommended (1) automatic rainfall exclusion with automatic simulant addition and (2) permanent exclusion cover systems for particular circumstances. The first system, automatic rainfall exclusion with automatic simulant addition, was used in a study by Shriner et al. (1985). The advantages and disadvantages of this method have been reviewed by Hogsett et al., (1986). These protocols are subject to the same requirements as described above for the first system described for wet deposition. Few additional changes in this document are needed to accommodate this protocol compared with the system which uses scheduled simulant additions. Documentation of volumes of simulated rain in real time as well as volumes of rain under ambient conditions must be recorded in automated simulant addition systems.

A permanent exclusion cover system may be used under certain circumstances. When a permanent cover is used, data to characterize the environment must be in place. Such environmental factors as air and soil temperature, relative humidity, light intensity (PAR), wind direction and velocity, and calculations of evapotranspiration must be made continuously at a minimum of one point per set of identical chambers, but more may be necessary depending upon variability.

2.4. DRY DEPOSITION - OPEN-TOP CHAMBERS

The International Workshop (Hogsett et al., 1986) recommended the use of open-top chambers, common to the National Crop Loss Assessment Network (NCLAN). Other research groups have used them as well. The technology is well developed and standardized (Heagle et al., 1973; Mandl et al., 1973;

Kats et al., 1974; Olszyk et al., 1980). In terms of quality control, all mechanical parts of such equipment must be maintained. The plastic should be cleaned frequently to maintain the highest quality light transmission. The plastic should be replaced when light transmission into the chamber is below 90% of a new panel. Chambers should be operated to provide at least three air changes per minute. One typical chamber will be monitored continuously for light transmission, air velocity, relative humidity, and pollutant concentrations. In this typical chamber, pollutant concentrations will be monitored continuously at two locations at canopy height. All filters will be checked daily during operation.

As a minimum, one gas inlet pipe for pollutant monitoring will be located in each chamber. These sample points should be located near the central portion of each chamber at canopy height. Automatic recordings of real-time data should be made available at all times. These chambers are subject to the same "alarms" as for the open-air protocol listed above. It must be recognized that air temperature and relative humidity are important determinants of plant growth and that, as with other systems, a restriction of air flow in these systems would result in elevated air temperature and relative humidity. Air temperature, light intensity, and relative humidity should be monitored continuously at the center of the chamber, and at canopy height in one chamber. Air flow rates must be maintained to the extent that the temperature in the chamber never exceeds 3°C above ambient conditions. If this limit is exceeded, the system should be reevaluated as soon as possible.

2.5. CONTROLLED-ENVIRONMENT FACILITIES

Many recent publications have focused on methods, timing, and data reporting, including units of expression of environmental condition for controlled-environment facilities (Berry et al., 1977; McFarlane, 1981; Spomer, 1981; Krizek and McFarlane, 1983). The guidelines of Krizek and McFarlane (1983) should be followed. These facilities are subject to the same types of monitoring and instrumentation quality assurance as all other types of facilities. The environments in such systems should be described in terms of temperature, relative humidity, light intensity in the PAR portion of the spectrum, and pollutant exposures. Pollutant exposures should not vary by more than 10% of set point and pollutant concentrations in all chambers should be monitored continuously on a time sharing basis. A strip chart recorder should be operated continuously with this monitor. Temperature, light intensity in PAR, and relative humidity should be monitored during fumigations in a sufficient number of chambers to guarantee that there are no significant differences among chambers.

2.6. GREENHOUSE FACILITIES

Quality assurance for greenhouse facilities is different from other previously described facilities. Such facilities do not provide the careful controlled environments of growth chambers or the ambient conditions of open field conditions. For quality assurance purposes, temperature, relative humidity, and light intensity in the PAR should be

monitored continuously. Periodic checks on background air pollutant concentrations, dust, etc. should be made. Temperatures are very important in experiments with forest species. An understanding of temperatures under ambient forest conditions may preclude a specific experimental approach. For example, temperatures in greenhouses should not exceed 90°F for studies with red spruce since this species rarely experiences this temperature in the forest. In many greenhouse facilities the only economical approach to cooling is provided by shading of various types. One unfortunate effect of shading is plant etiolation. It is specified that shading should not exceed 40% of PAR. It should be noted that the growing environment of controlled conditions can have profound effects on plant anatomy, physiology, and pollutant responses. In controlled environment facilities and greenhouse experiments, the most acceptable measure of pollutant concentration of exposure is the concentration measured in the exhaust line. The exposure containers should not change pollutant chemistry.

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3. PROTOCOL NARRATIVES FOR POLLUTANT DELIVERY AND MONITORING SYSTEMS

3.1. WET DEPOSITION

3.1.1. GUIDELINES

The following items should be used as guidelines for mixing, delivering, and monitoring wet depositions.

- Water to be used for simulated wet deposition should not be held in storage that influences its quality. Some justification is required to show that the container or the holding duration do not influence water quality.

- Only deionized or distilled water below 5 megohm conductivity should be used to make simulated precipitation. If the conductivity is above this limit, investigate and correct. The water used should be monitored for conductivity continuously.

- When simulants are being applied, the pH of the solution must be monitored continuously by at least one calibrated pH meter. Two pH meters are recommended. Differences between pH readings should be investigated and corrected. Calibrations should be made with a solution of relatively low specific conductivity.

- The distribution patterns and volumes should not vary by more than 10% of mean delivery volume per unit area within each plot among plots of the same treatment. Checks of patterns and volumes should be done once monthly, four areas per chamber, when the shelters are not over the treatment area.

- All nozzles from all plots should be removed and cleaned every two weeks of operation. Nozzles should be placed back into the system in a random manner.

- During each treatment application, grab samples from several nozzles should be monitored for pH and conductivity three times during life of stock solution.

- The mixing and distribution systems should remain closed, except to flush unwanted water, at all times so contamination from the environment cannot occur.

- Each pH meter must be calibrated with standardized buffers covering the pH range of test solutions prior to each day's treatments and the calibrations recorded in addition to usual testing.

- Two times per year, predetermined random grab samples of each acidity level should be obtained from nozzles and sent to an independent laboratory for analysis.

° The pH levels of the simulated wet deposition should be established by the PI after consulting with the particular Research Cooperative. The Forest Response Program should recommend pH levels to be used which are based upon assessment needs. The PI should have some recommended levels. Of prime importance is the number of treatments and the number of replicate plots of each treatment. The number of treatments is primarily determined by the method of statistical analysis used for assessment purposes. The number of plots per treatment should be maximized. At least 4 plots per treatment is required. At least 10 plots per treatment is strongly recommended.

3.2. DRY DEPOSITION

3.2.1. GUIDELINES

The generation of gaseous pollutants has been discussed briefly in a previous section. These systems are described in Table 2 (section 2.2). Each method has its benefits and limitations. The pollutants and the treatment regimes for each experiment can only be established by the PI (PIs) after consulting with the particular Cooperative Directors. Only treatments which reflect real-world conditions should be used in experiments to mimic natural conditions. Experimental exposures should be based upon exposure conditions that actually occur in nature. Therefore, treatments that implement (1) rapid changes in gas concentrations, (2) exposures to two gas pollutants that co-occur very rarely, or (3) unusually high gas concentrations should be avoided if possible. Under some circumstances, treatment levels outside these limits might be necessary to provide a more meaningfull dose-response function. Of prime importance is the number of treatments and the number of replicate plots of each treatment. The number of treatments is primarily determined by the method of statistical analysis. The number of plots per treatment should be maximized.

The assurance of quality from all tests with gaseous pollutants depends upon the accuracy of the analyzer/controller systems. The following narrative follows the general format of that used by the National Crop Loss Assessment Network (NCLAN).

When a large number of treatments are administered, time-sharing of air pollution monitors is a frequent occurrence. The sampling time of each area or unit should be 3 to 4 times longer than the minimum response time (see Table 2) to insure adequate monitoring. The International Workshop (Hogsett et al., 1986) encouraged a sampling frequency sufficient to provide characterization of an hourly average concentration. This situation is particularly relevant when monitoring SO_2 at temperatures below 20°C with relative humidities above 80%.

Under most gaseous exposure regimes it is necessary to mimic, as closely as possible, temporal variations that are normally experienced in polluted areas. For ozone exposures this would constitute relatively low concentrations between 1900 hrs and 0800 hrs daily. Between 0800 to

1900 hr daily, the O_3 concentration should increase, plateau, and eventually decrease.[3] The rate of increase and decrease as well as the duration of the plateau should follow ambient as much as possible. This protocol should be used in field applications and under most controlled-environment applications.

Under some circumstances that use controlled-environment applications, the protocol of a gradual increase and gradual decrease in gas concentration may be very impractical in terms of time, equipment, and financial support. Under these circumstances, a rapid increase and rapid decrease in gas concentration (i.e. square wave) may be used. Such protocols can be easily standardized and monitored, and the results are conveniently expressed. This latter protocol may be used in experiments of a preliminary nature such as screening plant species or various genetic isolines of a particular species, as well as determining, on a preliminary basis, dose-response relationships between pollutants and responses. In addition, such exposure protocols may be used for pollutants in which air quality data are poor. This protocol should not be used for assessment purposes.

Seventy-five percent of the total possible observations during a sampling period must be present to meet completeness requirements. The total observations possible will vary due to analyzer response time, lag times for time sharing systems, and duration of exposures.

3.2.2. QUALITY CONTROL CHECKS

3.2.2.1. ZERO AND SPAN CHECKS

Zero and span checks (OSC) will be used to assess data from automated and manual methods for precision. Each analyzer used to measure SO_2, O_3 and NO_x must have a weekly one-point zero and span check conducted with the values recorded on the zero and span data sheet.

Zero checks should be made by attaching the sample inlet line of the analyzer to a zero air source, such as an activated charcoal filter and silica gel, and allowing the analyzer to come to equilibrium and then recording the analyzer's output. Span checks should be made by attaching the sample inlet line of the analyzer to the appropriate outlet of a calibration source and allowing the analyzer to equilibrate and then recording the output. The concentrations of the calibration gas from the source should be in the mid-range of the expected level of pollutant to be measured. Using two check points within the experimental range instead of just the midpoint is recommended. Care must be taken to insure the outlet of the calibrator is vented to the atmosphere to avoid excessive back pressure. Also, the outlet flow from the calibrator must be greater than the inlet flow of the instrument to avoid dilution from the ambient air.

Changes or adjustments in either the zero or span settings after the initial startup of analyzers must be made when the reading obtained is greater than $\pm$ 5% of the known value of the zero or calibration gas. If this occurs, values on data sheets should be noted and brought to the

attention of the site project leader so that corrective action can be initiated immediately.

3.2.2.2 MULTIPOINT CALIBRATION

With a change of greater than ± 10% in the zero or span check (mentioned above), the operator should perform a multipoint calibration (MPC) on the analyzer, using at least four points including zero. If the slope of the new MPC curve differs significantly (approximately 10%) from the previous slope of the MPC curve, then the analyzer will be removed from service and repaired. However, it is first necessary to assure that differences in the MPCs are not caused by inaccurate readings, errors in transcribing, or in the calculations.

It is necessary to perform a multipoint calibration after an analyzer has malfunctioned, since the required repair may have affected the calibration. The MPC should be recorded in the analyzer's logbook along with the cause of the malfunction and the repair procedures. An MPC will be run at each startup of the analyzers. Calibrators and monitors should have an external calibration check and audit at least once annually on site.

3.2.2.3. CALIBRATION GAS SOURCES

Each site will have a calibration system for generating known levels of the pollutant gases. These systems will be used in making multipoint calibration curves and zero and span checks.

The gaseous standards, such as permeation tube devices and cylinders of compressed gases, that are used to obtain the test concentrations for SO_2 and NO_2 must be working standards traceable to a National Bureau of Standards gaseous Standard Reference Material (SRM) and used only within the certified period.

Test concentrations for O_3 can be supplied by the use of a UV lamp installed in a calibration system that has the capability of supplying O_3 to that analyzer in constant concentration at variable levels.

Test concentrations of the calibration gases must be supplied to the analyzer so that the analyzers are operating in their normal sampling mode. Test gases should pass through as much of the air line system as is practicable.

Verification of gas concentrations in the calibration cylinders should be accomplished every 12 months by sending the cylinders to the Performance Evaluation Branch of EPA's Quality Assurance Division of the Environmental Monitoring Systems Laboratory (at Research Triangle Park, North Carolina 27711) for analysis. Arrangements can be made by calling 919/541-2723 or FTS 629-2723.

3.2.2.4. AUDITS

Yearly external or independent internal audits will be used to assess data from automated and manual methods for accuracy. Independence is achieved by using standards and equipment different from those routinely used during calibration. It is preferred that audits be made by a different operator/analyst than the one conducting the routine analysis. If, however, circumstances dictate the routine operator/analyst also be the auditor, the individual conducting the audit must not be provided beforehand with the value of the standard used in auditing. Results of such audits will be examined carefully to detect any bias. All analyzers that measure SO_2, O_3, and NO_2 are audited in the same manner. That is, each analyzer is challenged with a known concentration of pollutant gas in concentrations at five levels including zero air. The differences between the known concentrations and the measured analyzer values are used to assess the accuracy of the monitoring data.

At least one audit should be conducted at each site during the growing season in which the data are being collected. The audit should be done approximately midway into the growing season. The acceptable accuracy level for analyzers in these audits will be ± 15 percent. This level is equivalent to the accuracy level established by EPA's Quality Assurance Division of the Environmental Monitoring Systems Laboratory at Research Triangle Park, North Carolina 27711.

Audit materials will be those furnished by the National Bureau of Standards (NBS SRM) or those that can be traced directly to SRMs. Calculations for the precision and accuracy of the measuring process will be done in accordance with the requirements as stated in the Federal Register, Vol. 43, No. 152, dated Monday, August 7, 1978, pages 34908 and 34909.

3.2.2.5. SAMPLING AND DATA COLLECTION SAMPLING SYSTEM

The sampling lines in the system will be inspected biweekly for foreign material and cleaned or replaced if necessary. Residence time of the sample gas must be less than 60 seconds. Sample inlet lines to the analyzers will consist of material that does not disturb the sample's integrity. Inlet sample filters are highly recommended. Losses of air pollutants in the system should be calculated bi-annually. Delivery rates should be adjusted to compensate for system losses. Flow meters should be checked twice annually. FEP (fluorinated ethylene propylene) is preferable over TFE (tetrafluoroethylene) due to the tendency of the TFE to absorb SO_2 and then degas after SO_2 is removed from the air stream.

3.2.2.6. EFFICIENCY TESTS

Constant concentrations of a series of test gas outputs from a certified source are used in determining sample system efficiency. A recently calibrated (same day) analyzer is used in conducting the sample system efficiency test in the normal sampling mode. The chamber's sample line is attached to the source and a known concentration of test gas is

generated and introduced into the sample line. The analyzer's response to this test gas is then compared to the known concentration. The percent difference between the site's analyzer output and the known concentrations of test gas will be calculated and reported to the project leader for each sample line in the system.

Other methods for determining the sample system efficiency may be acceptable provided the concentration of the test gas remains constant (±5%) throughout the tests and the concentration is at least eight times the minimum detection level of the analyzer. A discussion of these methods must be part of the quality assurance documentation kept at the site.

These tests should be done at least prior to the start of the exposures and after the completion of the study, or every six months, whichever is less.

3.2.3. DATA REPORTING

The project leader, prior to data collection, will write procedures for data collection and reporting to insure the comparability of the data with other sites. Data collection from those analyzers and instruments that are connected to a data acquisition system will be in a format comparable with the research project as specified by the project leader. Data will be reported in engineering units on those systems which have the capability of converting voltage to the desired units. The engineering units for the various parameters are as follows:

Ozone, SO_2, NO_x.....ppm (altitude influences may be significant)

Solar radiation.....μmol cm^{-2} s^{-1}

Temperature.....°C

Humidity.....% relative humidity

Wind speed.....m s^{-1}

Wind direction.....degree azimuth.

3.2.3.1. DATA COLLECTION FLOW CHARTS

Data flow charts giving the data collection format used at the different sites should be standardized by the Forest Response Program Cooperatives.

3.2.3.2. DATA VALIDATION

The senior scientist at each site will be responsible for data validation. Data sheets, maintenance records, strip chart recording, and other pertinent data will be examined for:

- high or low values (outliers);
- rapid excursions, such as caused by electronic interference;
- repetitious values, such as caused by equipment malfunction;
- time continuity; and
- completeness, at least 75% of the data available from the sampling system in use.

The following references for data validation are available from CERL upon request: Quality Assurance and Data Validation for the St. Louis Regional Air Pollutant Study written by R.B. Jurgens and R.C. Rhodes, and Screening Procedures for Ambient Air Quality Data, EPA-450/2-78-037 (OAQPS 1.2-092), July 1978.

3.2.4. ANALYZER METHOD REQUIREMENTS

All analyzers used in the project for measuring pollutant levels in the ambient atmosphere must be a Reference Method or have met the equivalency requirements as specified by EPA in the Federal Register, 40 CFR 53. Any modifications made to the air pollutant analyzers that disqualify them as reference or equivalent methods (e.g., changes made in order to perform special purpose monitoring as required by the research project) must be noted in the instrument logbook.

3.2.5. AMBIENT AIR ANALYZERS - OPERATION AND MAINTENANCE

1. Operation procedures will be those specified in the manufacturer's operators manual.
2. Manufacturer's operators manuals will be available for each type analyzer at each location.
3. Measurement principles and interferences caused by CO, H_2S, and heated silver scrubbers are discussed in section 3 of Document No. QAD/M-79.12: Summary of Performance Test Results and Comparative Data for Designated Equivalent Methods for SO_2. This document is available upon request from the Quality Assurance Officer at CERL.
4. A preventive maintenance schedule will be followed by the operators for each analyzer, instrument, and system used in the collection of data for the project.
5. An example of the forms will be included in each analyzer's operators manual. They indicate what needs to be done and the frequency.
6. To insure implementation of the preventive maintenance schedule, the forms will be filled out by the operator and a copy sent to

the QA Officer, and the Project and/or Field Leader who will have the responsibility of assuring the maintenance is being performed. Originals are to be stored on site.

7. A supply of spare parts required for routine maintenance of each analyzer, instrument, and system will be maintained on site to insure that downtime is held to a minimum.

8. A logbook for each analyzer, instrument, and system will be maintained and all malfunctions, repairs, multipoint calibrations, modifications, etc. will be noted.

3.3. REFERENCES

Jurgens, R.B. and R.C. Rhodes. 1976. Quality Assurance and Data Validation for the St. Louis Regional Air Pollutant Study. In: Proceedings of the Conference on Environmental Modeling and Simulation, W. R. Ott, ed. EPA 600/9-76-016.

U.S. Environmental Protection Agency. 1978. Screening Procedures for Ambient Air Quality Data. EPA-450/2-78-037 (OAQPS 1.2-092).

Summary of Performance Test Results and Comparative Data for Designated Equivalent Methods for SO_2. 1979. QAD/M-79.12. (Complete reference unavailable.)

4. STANDARD OPERATING PROCEDURE FOR MEASUREMENT OF NET CARBON EXCHANGE

4.1. SCOPE AND PURPOSE

Several previous studies have indicated that atmospheric pollution impacts the physiological processes involved in net carbon exchange (NCE) and may ultimately reduce forest productivity. Measurements of NCE can help identify:

- the extent to which photosynthesis and respiration are impacted by dry and wet deposition and
- can provide information to use in estimating the growth loss that may result from pollutant damage to the physiological processes involved in NCE and leaf area dynamics.

This SOP will outline the minimum SOPs for relating NCE of seedlings grown in growth chambers or open-top chambers to (1) NCE capacity at specified environmental conditions and (2) to relate measures of NCE and leaf area to seedling growth rate.

The NCE measurements considered are those necessary to meet the objectives outlined in the 1986 Forest Response Program.

4.2. MATERIALS AND SUPPLIES

4.2.1. EQUIPMENT

Any NCE equipment which meets the specifications given in Appendix B may be used. Portable units commercially available include the LI-COR LI-6000 and ADC-LCA2. If response curves for NCE to carbon dioxide concentration, light, temperature and relative humidity are required, cuvettes with the capacity for manipulating these elements will be required. It will be the responsibility of the operator to be sure the appropriate chamber is selected so that leaf temperature elevation, CO_2 depletion, and boundary layer resistance are maintained within the specified limits given in Appendix B.

4.2.2. CHEMICALS/REAGENTS

- Primary standard gases 0, 350, and 1000 ppm that are traceable to NBS standards
- Soda lime
- Magnesium perchlorate

4.3. PROCEDURES

NCE measures are instantaneous readings which are not amenable to precision and accuracy statements and for which no standards are available. Quality assurance largely depends on calibration and spot checks during equipment operation to ensure the equipment is functioning properly. This section deals with what plant material should be measured, the frequency of measurements needed for two specific type studies, and what associated environmental variables should be measured so that data can be compared across locations.

In general, the operator should follow the manufacturer's standardization checks and procedures (Appendix B) to ensure proper operation and maintenance of instruments used in making the NCE and associated environmental measurements.

4.3.1. SAMPLE PREPARATION

- Use random selection procedures to select trees and branches for measurement of NCE as dictated by experimental design.
- Select foliage from each morphological class (flush, level of maturity, age) as dictated by experimental design.
- Mark foliage to be enclosed in the chamber so it can be reused for the remainder of the day. This is required to minimize the amount of leaf area determination that has to be made.

4.3.2. EQUIPMENT OPERATION

4.3.2.1. MEASUREMENT LOCATION ON SELECTED FOLIAGE

4.3.2.1.1. Long Needle Conifers-Pine Like

Center the cuvette on the selected needles as much as possible if the entire needle is not enclosed in the chamber.

4.3.2.1.2. Short Needle Conifers-Spruce Like

Center cuvette on branch containing foliage in the age class to be measured.

4.3.2.1.3. Hardwoods

Center the cuvette on the side of leaf to be measured.

4.3.2.1.4. Alternative

Entire seedling may be enclosed in the chamber. In this case, and in the case of short needle conifers, the amount of stem and branch material enclosed in the cuvette should be determined.

4.3.2.2. CHAMBER FOLIAGE CONSIDERATIONS - FOLIAGE QUANTITY AND PLACEMENT IN THE CHAMBER

4.3.2.2.1. Portable Units

Long Needle Conifers-Pine Like

1. Use 2-5 needles (one fascicle) - this will depend on pine type.

2. Place needles so they do not run diagonally across the chamber if needle length is to be assumed to be equal to the length of the chamber.

3. Place needles in the cuvette so they do not overlap.

4. Place needles so they do not lay against each other.

5. Program instruments with a built-in memory with an estimate of the actual needle surface area to be enclosed in the chamber. This will help the operator to know if the instrument is within an expected range of operation.

Short Needle Conifers-Spruce Like

1. The length of the chamber and needle length and frequency will dictate the amount of foliage that is included in the chamber.

2. Caution: Boundary layer resistance problems can be created when large amounts of foliage are used (see Appendix B).

3. Program the equipment memory with a fixed value of leaf area and adjust carbon exchange rates as soon as leaf area is determined.

Hardwoods

1. It is best to use a chamber which provides a fixed leaf area inside the chamber.

2. Program the equipment memory with the actual leaf area enclosed inside the cuvette.

4.3.2.2.2. Controlled Environment-Large Cuvette Units

° The amount of foliage enclosed in the cuvette will depend on the cuvette size and flow rates used.

° Placement should be such that foliage overlap and self shading are minimized and conditions specified in Appendix B are met.

4.3.2.3. DETERMINATION OF LEAF AREA ENCLOSED IN THE CUVETTE

This section outlines the steps recommended for determining leaf area using either destructive or non-destructive techniques. Whether a destructive or non-destructive technique is used will depend on the available plant material and whether the NCE measurement coincides with a planned destructive harvest. The actual procedures for determining leaf area are covered in the SOP for Measurement of Seedling Leaf Area (section 6.3). The technique selected for determining cuvette leaf area must meet the specification outlined in Appendix B and follow the QA checks outlined in the SOP for Measurement of Seedling Leaf Area.

4.3.2.3.1. Non-Destructive Method

Non-flat needle Conifers

1. Determine the number of needles in the cuvette for each sample.
2. Determine the length of all needles enclosed in the cuvette.
3. Determine the diameter on a subsample of needles.
4. Develop a length and/or width equation for predicting area of all needles.
5. Estimate leaf area from an equation relating leaf area to needle length and/or width measurements.

An alternative to steps 3, 4, and 5 is to relate a single dimension measurement (e.g., length) to volume displacement and then calculate total surface area. To use either of these techniques, an R^2 of .90 or better should be obtained for the relationship between the measured dimension variable(s) and measured leaf area.

4.3.2.3.2. Destructive Method

Non-flat needle Conifers

1. Harvest foliage enclosed within the cuvette.
2. Determine leaf area by a volume displacement method.

Hardwoods and Flat-needle Conifers

For hardwoods and flat-needle conifers, projected leaf area will be determined by a planimetric technique such as with the LI-3000, LI-3100, or Delta-T devices.

4.3.2.4. FREQUENCY OF MEASUREMENT DATES

The actual number of days between measurements of NCE cannot be specified for all study objectives and conditions. However, some

consideration should be given to the maximum number of days that should be permitted to elapse before NCE measurements are repeated for two general objectives that are of major importance to the Forest Response Program. These two objectives are (1) determination of the impact of pollutant exposure on the physiological processes involved in NCE and (2) determination of the relationship between pollutant exposure NCE and seedling growth rates.

4.3.2.4.1. For Studies with the General Objective of Relating Pollutant Exposure to Impacts on the Physiological Processes Involved in NCE

For pollutant exposure studies conducted in growth chambers and that are less than four months in duration, NCE should be measured (1) prior to the beginning of the experiment and (2) at the end of the experiment. Two general conditions should be met for these measurements of NCE:

1. Prior to measurement, the seedlings should be given at least one week to adjust to the environmental conditions under which NCE is to be determined (e.g., NCE should not be determined at full sunlight if the seedlings are kept in a facility where they normally receive only one-half full sunlight).

2. Environmental conditions (CO_2, light, temperature) should be the same for both measurement dates if the investigator is interested in determining the change in NCE capacity over the exposure period.

For exposure periods longer than four months, NCE should be measured every four months and at the end of the experiment. Measurement considerations are the same as given for the short term studies. In the case of open-top chamber studies, measurement conditions will not be the same for each measurement date.

4.3.2.4.2. For Studies with the General Objective of Determining the Effect of Pollutant Exposure on NCE and Relating Changes in Whole Plant NCE to Seedling Growth Rates

Net carbon exchange must be measured at a minimum on a two week basis.

4.3.2.5. FREQUENCY OF MEASUREMENTS WITHIN A MEASUREMENT DATE

4.3.2.5.1. For Studies with the General Objective of Relating Pollutant Exposure to Impacts on the Physiological Processes Involved in NCE

Measure NCE at one time period during the photoperiod. This should be when light, temperature, and CO_2 are closest to their optimum value that is provided by the facility at which the study is being conducted.

Measure NCE at one time during the night period after the seedlings have been in the dark for at least one hour and temperature has been stable for one hour.

4.3.2.5.2. For Studies with the General Objective of Determining the Effect of Pollutant Exposure on NCE and Relating Changes in Whole Plant NCE to Seedling Growth Rates

NCE potential should be measured over the entire range of light, temperature, and relative humidity conditions that can occur at each measurement date. This can be accomplished two ways:

1. by constructing light and temperature response curves while controlling relative humidity, or

2. by measuring NCE on both cloudy and clear days at 3 hour intervals throughout the day.

Note: Both approaches take considerable amounts of time, and it is unlikely that more than 1 or 2 families could be considered for this type study. It will require that a minimum of 5 trees be measured for each treatment-family combination.

4.3.2.6. REQUIRED ENVIRONMENTAL, PLANT, AND INSTRUMENT MEASURES TO BE TAKEN WITH EACH SAMPLING PERIOD WITHIN A DAY

- Ambient CO_2 concentration
- Absolute chamber CO_2
- Leaf temperature (This will not be possible with conifers - record chamber air temperature.) Note: caution given in Appendix B.
- Air temperature
- Chamber relative humidity
- Air relative humidity
- Photosynthetically active radiation
- Stomatal conductance
- Flow rate
- Barometric pressure

4.4. PREVENTIVE MAINTENANCE

The following guidelines apply to the cuvette and the infra-red gas analyzer.

- Use and store per guidelines given in the operators manual provided by each company.
- Do not leave exposed to direct sunlight when not in use.
- Do not store under environmental conditions that fall above or below the operating range of the instrument or that vary widely.
- Do not use when weather is misty or raining - carry a protective covering with you to cover the instrument if it begins to rain.
- Do not lay chamber on rough surfaces or it will get scratched and light transmittance properties will decrease.

4.5. CALIBRATION PROCEDURES

4.5.1. CO_2 ANALYZER

- Factory calibrate annually.
- Zero check and readjust hourly during measurement sessions.
- If zero drift is >5 ppm in three consecutive hourly checks, return the instrument to the site supervisor for repair.
- If a zero drift of >5 ppm occurs, requiring adjustment, recheck and readjust the span check also before continuing to use the instrument.
- Span check at the beginning of each measurement day using primary standard gases with 0 (N_2) and 350 ppm (CO_2 in N_2) that are traceable to NBS standards.

4.5.2. QUANTUM SENSOR (LI-190 S-1)

- Factory calibrate annually.
- Keep protective cover on the sensor when not in use.

4.5.3. FLOW METER

- Factory calibrate annually.
- Check every four months with a high quality rotometer mounted in series with the air stream entering the cuvette. If they disagree by more than 6%, the site supervisor should be notified and repairs made before the instrument is reused.

4.5.4. RELATIVE HUMIDITY SENSOR

- Factory calibrate annually.

- Spot check monthly with air of two known vapors (high and low humidity) within the operating range of the instrument. A dew point hygrometer that has been calibrated within the measurement year or spot checks kits available from LI-COR for the LI-1600 may be used. If the measured values differ by more than 5% but less than 10% from the standard values, readjust the instrument and record the adjustment in the log book. If the deviation is greater than 10%, then (1) replace the sensor and recalibrate or (2) send to the factory for recalibration.

4.5.5. CUVETTE THERMOCOUPLE OR THERMISTORS

- Factory calibrate annually.

- Compare readings at a high and low temperature with a high quality thermometer that has been calibrated by the water bath technique on a six month interval. If readings differ by more than ±2°C, then readjust and recalibrate the instrument.

- For units with a thermistor housed within the unit and in the cuvette chamber, a comparison of the thermocouple readings when the chamber is open and the fan running is a good field check.

- All calibrations performed should be recorded, dated, and signed in an instrument log book. This does not include field spot checks.

4.6. CALCULATIONS/UNITS

4.6.1. CALCULATIONS

- Calculations are to be done as recommended by the manufacturer's manual. In the case where no manual is available, refer to pp. 50-54 and pp. 162-166 in Plant Photosynthetic Production - Manual of Methods by Sestak, Catsky and Jarvis (1971) for NCE calculations. For conductance calculation of CO_2, O_2, and O_3, follow the procedures outlined by Coombs et al., (1985) using the appropriate diffusivity coefficients (refer to pp. 85-87).

- Calculations should be done on a per leaf area and an oven dry weight basis. Leaf area for conifers will be that of the total surface area. Leaf area for hardwoods will be that for one surface. If stomatal conductance of the adaxial surface is >.04 cm/s it should be reported in the written report for the study.

4.6.2. UNITS

Apparent net photosynthesis (NCE).....CO_2 $\mu mol\ m^{-2}s^{-1}$

Dark respiration.....CO_2 $\mu mol\ m^{-2}s^{-1}$

Leaf Area
Conifers.....cm^2 (total surface)

Hardwoods.....cm^2 (one sided)

CO_2 concentration.....ppm

Temperature.....°C

Photosynthetically active radiation.....µmol $m^{-2}s^{-1}$

Transpiration.....µmol $m^{-2}s^{-1}$

Stomatal conductance.....µmol $m^{-2}s^{-1}$

Relative humidity.....%

Leaf area.....cm^2

Barometric Pressure.....KPa

4.6.3. RECORDING FORMAT (see Appendix D for suggested computer codes and reporting symbols).

Date.....0-365

Time.....Standard Military

Treatment .....Number

Tree .....Number

Branch-Sample .....Number

Foliage age class.....Oldest=1, Next Age=2, Etc.

Sample leaf area.....Nearest hundredth cm^2

Programmed value.....Nearest hundredth cm^2

Actual value.....Nearest hundredth cm^2

Flow rate.....l/hr

Ambient CO_2.....Nearest ppm

Chamber CO_2.....Nearest ppm

Leaf temperature.....Nearest tenth °C

Air temperature.....Nearest tenth °C

Chamber relative humidity.....Nearest percent

Air relative humidity.....Nearest percent

Photosynthetically Active Radiation.....Nearest µmol $m^{-2}s^{-1}$

Carbon exchange rate (NCE).....Nearest µmol $m^{-2}s^{-1}$

Internal CO_2 concentration.....Nearest ppm

Transpiration rate.....Nearest µmol

Stomatal conductance.....Nearest µmol

4.7. ERROR ALLOWANCE AND DATA QUALITY

4.7.1. EQUIPMENT PRECISION AND ACCURACY REQUIREMENTS

Instrument/Procedure	Accuracy	Precision
Quantum sensor	±5%	±2%
Humidity sensor	±5%	±2%
Temperature sensor	±1°C	±1°C
CO_2 analyzer	±5 ppm	±2 ppm
Noise	< 1%	N/A
Area meter	±5%	±5%
Scales/oven dry weight	±1.5%	±1.5%

All instrumentation will be checked to ensure that precision and accuracy are within the acceptable range. Precision measures for the CO_2 analyzer and associated measures are to be made at the time of calibration under controlled conditions.

4.7.2. DATA QUALITY OBJECTIVES

Variable	Reporting Units	Repeated Measurement Error at Lower Limit	Repeated Measurement Error at Upper Limit	Measurement Accuracy Tolerance
Photosynthesis	$\mu mol\ m^{-2}s^{-1}$	± 10%	± 10%	15%
Transpiration	$\mu mol\ m^{-2}s^{-1}$	± 10%	± 10%	15%
Needle Conductance	$\mu mol\ m^{-2}s^{-1}$	± 10%	± 10%	10%
Respiration	$\mu mol\ m^{-2}s^{-1}$	± 10%	± 10%	15%

4.8. REFERENCES

Coombs, J.D., D.O. Hall, S.P. Long, and J.M.O. Scurlock (eds.). 1985. Techniques in Bioproductivity and Photosynthesis. Pergamon Press, New York. 298 pp.

Sestak, Z., J. Catsky and P.G. Jarvis. 1971. Plant Photosynthetic Production - Manual of Methods. W. Junk Publications, The Hague.

5. STANDARD OPERATING PROCEDURE FOR PLANT WATER RELATIONS MEASUREMENTS

5.1. SCOPE AND PURPOSE

Plant water relations measurements are important measures to include in the Forest Response Program (FRP) because they (1) serve as a good index of plant vigor and (2) are a necessary component in estimating the amount of atmospheric pollutant that was taken in by the plant under study. The two plant water relations measures to be addressed in this SOP are (1) measures of xylem pressure potential (XP) and (2) measures of stomatal conductance (i.e., leaf water vapor exchange). The procedures described will be for (pine) seedlings used in growth chamber or open-top chamber studies as outlined for the Forest Response Program.

5.2. MATERIALS AND SUPPLIES

5.2.1. EQUIPMENT

5.2.1.1. XYLEM PRESSURE POTENTIAL DETERMINATION

- Pressure Chamber - The pressure chamber is the recommended equipment for determining xylem pressure potential. Two available sources are PMS Instruments and Soil Moisture Test.
- Dry nitrogen source and dual stage pressure regulators
- Tank wrenches
- Rate valve adjustment wrench
- High intensity light source for pre-dawn measurements; also extra batteries
- A 10X or better magnifying glass; alternatively, a binocular scope mounted on the pressure chamber
- Industrial grade razor blades
- Zip lock plastic bags
- Paper towels
- Plastic wrap
- Tissues
- Water
- Stem inserter
- External light source and extra batteries and bulbs

5.2.1.2. LEAF WATER VAPOR EXCHANGE DETERMINATION

- Porometer - The recommended method of measurement of stomatal conductance is by the steady state porometer technique. Instruments such as the LI-1600, Delta-T MK3 porometer, LI-6000, and LCA-2 have the capability of making these measurements.

- Cuvette Chambers - The cuvette chamber should meet the specifications outlined in Appendix B. For pine-like foliage, a small volume square chamber has several advantages: (1) readings can be taken on a single fascicle - this makes foliage placement into the cuvette easy and fast; (2) the length of the chamber can be taken as the length of the needle; (3) the response time of a small volume chamber will be fast, thus minimizing temperature, relative humidity, and CO_2 departure from ambient; (4) only small amounts of foliage are required, thus keeping the amount of foliage for which leaf area has to be determined to a minimum.

- Desiccant such as silica gel

- Extra batteries

5.2.2. CHEMICALS/REAGENTS

5.2.2.1. XYLEM PRESSURE POTENTIAL DETERMINATION

- Alcohol for cleaning resin on equipment

- Vaseline for greasing O-rings

5.2.2.2. LEAF WATER VAPOR EXCHANGE DETERMINATION

- Silica gel

5.3. PROCEDURES

5.3.1. SAMPLE PREPARATION

5.3.1.1. XYLEM PRESSURE POTENTIAL DETERMINATION

5.3.1.1.1. Sample Marking

1. Set pressure chamber up as close to seedling to be measured as possible.

2. For predawn determinations, select and mark on the day prior to the measurement day the foliage sample is to be used. The marking should contain the key sample identification information. When sample is collected put ID tag with the sample.

5.3.1.1.2. Sample Collection

1. If vapor pressure deficits are greater than 10 mbar (.001 MPa), wrap the foliage to be removed with plastic wrap just prior to cutting it off. This will prevent rapid changes in XP from occurring before determinations can be made.

2. Remove the petiole, fascicle, or branch from the seedling.

3. If petioles or branches are used for XP determination, they should be cut at a 45° angle with a sharp razor blade free of resin. The 45° angle creates a surface that is easier to illuminate and detect the end point. A sharp clean razor blade will keep the petiole or stem from splitting during the excision process. If stems are to be used, cut the stem so that a distance of 3 cm is left between the cut surface and the first lateral branch.

4. If fascicles are to be used, they should be cut as close to the stem source as possible. On some species it may be better to tear the fascicle off; this leaves the vascular trace intact.

Note: Accuracy and precision considerations -- Accuracy and precision will be greater for stem material than for fascicle than for individual needles. This is especially true for the predawn determinations of XP. The available study material will dictate what material can be used. Determination of XP on individual needles is not recommended due to high sample to sample variation.

5.3.1.1.3. Storage of Samples

1. In some studies it may be possible to cover the seedlings with a bucket to extend the time period available for predawn XP determination.

2. In other studies it will be necessary to collect multiple samples and transport the material for XP measurements. If sample has to be transported or held for more than 30 seconds after cutting, it should be placed in a zip lock bag (or similar device) which contains a moist paper towel (no excess water). All excess air should be squeezed out of the bag and then the bag sealed. Store the bag in a cool spot out of direct light. Storage time should be less than 5 minutes. If longer storage times are required, a study which quantifies the magnitude of change in XP that occurs in storage over the length of storage period required should be made and measured XP adjusted for length of storage time.

5.3.1.2. LEAF WATER VAPOR EXCHANGE DETERMINATION

1. Mark foliage to be used for water vapor exchange. This will be necessary so foliage leaf area can be determined and to locate the

sample of repetitive measures within the day or to be made on the same foliage.

2. Sample selection procedures should be the same as those outlined in the SOP for Net Carbon Exchange (section 4.3).

3. If samples are to be collected and stored for leaf area determination, they should be placed in a zip lock bag and placed in the refrigerator. A tag with all key identification information must be placed inside or on each bag. Samples should not be stored more than 2-3 days or decomposition may be significant.

5.3.2. EQUIPMENT OPERATION

5.3.2.1. XYLEM PRESSURE POTENTIAL DETERMINATION -- PRESSURE CHAMBER

1. Collect foliage as per Section 5.3.1.

2. On stem material, peel the bark back about 2.54 cm to prevent "foaming"-material from the phloem-cambium area from bubbling onto the cut surface. Do not tear any small lateral branches off the sample in this step.

3. Insert sample material into the chamber stopper or holder. Use an inserter where necessary. If the stem or fascicle is inadvertently bent sharply do not use it. Note, under vapor pressure deficits greater than 10 mbars (.001 MPa), keep the material wrapped until the insertion step is complete.

4. For stems, insert the peeled stem through the stopper and lid so that ≈ 6 mm of stem is above the upper lid surface.

5. Insert sample and pressurize the chamber.

6. Begin with chamber valve off and rate valve off.

7. Put stopper with sample inserted through it into the chamber lid.

8. Remove plastic wrap from the foliage and fasten lid securely on the chamber as quickly as possible.

9. Switch the chamber valve to the fill position.

10. Adjust rate valve to pressurize the chamber at .05 MPa (.5 bars)/second. For predawn XP measurements when soil moisture is high, a rate about .025 MPa/s may be desirable. When it has already been established by previous measurements that XP is low, a faster pressurization is permitted until a reading within $\geq$ .5 MPa is obtained. Then the rate should be sloved to 0.05 MPa/s.

11. For species with resin ducts such as the pines, during the pressurization process it will be necessary to periodically wipe the resin off the cut surface with a tissue that has a high absortivity capacity.

12. View end point through a 10X or greater magnifying glass or scope. The end point is when the xylem surface first wets.

13. Turn chamber valve to off.

14. Turn rate valve off.

15. Turn chamber valve to exhaust.

16. Let gauge pressure go to zero.

17. Remove lid. Turn chamber valve to off.

18. Dispose of sample.

Note: Accuracy and precision considerations -- For plant water relations, the thermocouple psychrometer is the accepted standard of measure for plant water potential. It would be possible to compare xylem pressure potential measurements to plant water potentials as measured with the thermocouple psychrometer. However, since the objective of plant water relations measures outlined for the FRP is to obtain an index of plant moisture states, this is not justified. Thus no measure of accuracy will be made. A measure of precision can be made by taking repeated measures of xylem pressure potential using tissue from a single branch and using plant material as similar as possible. This measure of precision will still have a within-plant variation component in it as well as a machine repeatability component. Precision of XP determinations should be made for each species across the full range of xylem pressure potentials that are experienced during the study.

5.3.2.2. LEAF WATER VAPOR EXCHANGE -- POROMETER/CUVETTE

For foliage selection, measurement location, and determination of cuvette leaf area, see Net Carbon Exchange SOP (Section 4.3.1.-4.3.2.3.)

5.3.2.2.1. Frequency of Measurement Dates

This will be dictated by the rate of change of factors which are known to influence stomatal functioning: (1) foliage development, (2) vapor pressure deficits, (3) light levels, and (4) soil moisture supply.

5.3.2.2.2. Porometer Operating Procedures: Checkout

1. Prior to each day's use of the porometer, freshly dried desiccant is installed.

2. The system should then be checked for leaks by setting the flow to zero.

3. Valves are then checked to see if they will fully open by turning the flow meter to the fully open position.

5.3.2.2.3. Porometer Operating Procedures - Measurements

1. Operate per operators manual instructions (Appendix B).

2. With hardwoods, make sure the thermistor or thermocouple is touching the lower surface of the leaf.

3. With conifers, make sure the thermistor or thermocouple is not exposed to direct sunlight.

4. Associated light readings should be taken with the Quantum sensor (held level).

5. Stomatal conductance readings should be made at a chamber relative humidity that is within ±5% of that observed for the ambient air.

6. Under high temperature and radiation conditions it may be necessary to shield the chamber to keep chamber temperature during the reading within ±2°C of ambient air temperature.

7. For open-top studies, if dew is present it will not be possible to measure stomatal conductance until the foliage has completely air dried.

Note: Accuracy and precision considerations -- It is not possible to measure the same foliage and get an estimate of the precision of the porometer measures because stomatal response to repeated measurements does occur. Both the precision and accuracy determinations will have to be made during, and will depend on, proper calibration, operating procedures, and equipment maintenance.

5.3.3. REQUIRED PLANT AND ENVIRONMENT MEASURES FOR ALL PLANT WATER RELATIONS STUDIES

- Air temperature
- Relative humidity
- Leaf temperature (for hardwoods)

- Light intensity - net radiation or photosynthetically active radiation.

- Soil water potential - optional but desirable

Use air temperature and relative humidity to calculate vapor pressure deficit for conifers. Use leaf temperature and relative humidity to calculate vapor pressure gradient for hardwoods. For chamber studies or measurements made below the canopy, use the Quantum sensor.

5.4. PREVENTIVE MAINTENANCE

5.4.1. PRESSURE CHAMBER

- Clean foliage and grit from around the chamber lid.

- Keep all hose and quick-disconnect fittings free of grit.

- Keep the O-rings free of resin and greased with a lubricant.

- Do not run the rate valve down hard against the valve seat.

- Do not leave foliage in the chamber during storage. Make sure the chamber is cleaned and lubricated before storage.

- Store the magnifying glass where it will not be scratched. Clean all resin from the lens before storing.

- Remove batteries from flashlights before storing.

- Dispose of all used razor blades at the end of the measurement day.

- Store pressure regulator in a clean environment.

5.4.2. POROMETER

Storage procedures are the same as those given for the LI-6000 in the SOP for Net Carbon Exchange (Section 4.4) and provided by the manufacturer (Appendix C).

5.5. CALIBRATION PROCEDURES

5.5.1. PRESSURE CHAMBER

- No annual calibration is required.

- Pressure gauge should be observed for jerky movements during the pressurization process. If this occurs, have it replaced. The pressure gauge should be checked to see that it returns to zero when the pressure is released. If it does not, adjust the gauge to properly read zero.

5.5.2. POROMETER

- An annual company calibration is required. The tests performed in this calibration is given in Appendix E.

- Spot checks of the humidity sensor, Quantum sensor, and thermistor are the same as those given in the Net Carbon Exchange SOP sections 4.2.-4.5.and Appendix B.

5.6. CALCULATIONS/UNITS

Calculations of stomatal resistance, conductance, and transpiration should be made as described in the porometer owner's manual.

5.6.1. UNITS

Stomatal conductance.....$\mu mol\ m^{-2}s^{-1}$

Stomatal resistance.....$\mu mol\ m^{-2}s^{-1}$

Transpiration.....$\mu mol\ m^{-2}s^{-1}$

Quantum flux density.....$\mu mol\ m^{-2}s^{-1}$

Air temperature.....°C

Leaf temperature.....°C

Relative humidity.....%

Vapor pressure deficit or gradient.....mg/m

Soil water potential.....MPa

5.6.2. RECORDING FORMAT (See Appendix D for suggested computer codes and reporting symbols.)

Date.....0-365

Time.....Standard Military

Treatment.....Number

Seedling.....Number

Sample.....Number

Xylem pressure potential.....MPa-nearest hundredth

Stomatal conductance.....nearest μmol

Stomatal resistance.....nearest μmol

Transpiration.....nearest μmol

Quantum flux density.....nearest μmole

Air temperature.....°C-nearest tenth

Leaf temperature.....°C-nearest tenth

Relative humidity.....%-nearest tenth

Vapor pressure deficit or gradient.....mg m^{-3}-nearest tenth

Soil water potential.....MPa-nearest hundredth

Cuvette leaf area

Hardwoods.....nearest tenth cm^2 (one sided measure)

Conifers.....nearest tenth cm^2 (total surface)

5.7. ERROR ALLOWANCE AND DATA QUALITY

5.7.1. XYLEM PRESSURE POTENTIAL DETERMINATION

The major source of error in determining xylem pressure potential is in reading the "end point." This error can be minimized by (1) following the steps outlined in the operating procedure section (5.3.2.1.) of this SOP and (2) providing appropriate training of new users before they are allowed to make study measurements. The accepted precision for xylem pressure potential determinations is $\pm$0.1 MPa.

5.7.2. LEAF WATER VAPOR EXCHANGE DETERMINATION

The error sources of steady state porometers arise from measurements of temperature, relative humidity, flow rate, and leaf area. The acceptable limits are the same as those given in Appendix B.

5.7.3. WATER RELATIONS MEASUREMENTS - DATA HANDLING

The QA procedures outlined for NCLAN biological measurements are acceptable for the Forest Response Program (Appendix F).

5.7.4. DATA QUALITY OBJECTIVES

Variable	Reporting Units	Repeated Measurement Error at Lower Limit	Repeated Measurement Error at Upper Limit	Measurement Accuracy Tolerance
Leaf Water Potential	MPa	± 0.1%	± 0.1%	5%
Leaf Water Content	% wt	± 1.5%	± 1.5%	1%
Leaf Area	0.01 cm^2	± 2%	± 5%	5%
Needle Conductance	µmol $m^{-2}s^{-1}$	± 10%	± 10%	10%

5.8. REFERENCES

N/A

6. STANDARD OPERATING PROCEDURE FOR MEASUREMENT OF SEEDLING LEAF AREA

6.1. SCOPE AND PURPOSE

Measures of leaf area are necessary for calculating net carbon exchange and water vapor exchange on a per unit leaf area basis. Estimation of whole plant gas exchange and pollutant uptake requires combining unit area rates of gas exchange with measures or estimates of seedling leaf area. This SOP will address the procedure necessary for determining leaf area by the planimetric and volume displacement methods.

6.2. MATERIALS AND SUPPLIES

6.2.1. EQUIPMENT

- vernier caliper
- analytical balance (e.g., Mettler A30)
- thin wire
- leaf area meter

To determine leaf area using planimetric methods, several leaf area meters can be used to give quick, reliable results with high resolution. LI-COR, Inc., produces the LI-3100 Area Meter. It is an instrument with interchangeable 1.00 mm^2 and 0.1 mm^2 resolution capability with a 35 mm and 105 mm lens. A 25 cm wide sample guide is provided for the 1.0 mm^2 resolution configuration. A 7.5 cm wide sample guide is available for the 0.1 mm^2 resolution capability (LI-COR, 1979). Portable leaf area meters such as the LI-3000 are available from LI-COR, Inc., for non-destructive sampling and field work.

Another leaf area meter such as the LI-3000 used for studies in the Forest Response Program is the Delta-T, distributed by Decagon Devices, Inc. This system (Delta-T Devices, Ltd.) can measure the area of all shapes and sizes of leaves, from large maple leaves and long cereal leaves to small pine needles. Measurements of diseased and variegated leaf area are possible if the discolored part is in good contrast with the remainder of the leaf. Resolution of 1/300th of the scanned width and height is given. Ranges from 1 mm^2 for large areas (360 x 260 mm), down to 0.01 mm^2, for small areas (35 x 26 mm).

6.3. PROCEDURES

6.3.1. SAMPLE PREPARATION

6.3.1.1. GENERAL CONSIDERATIONS FOR SAMPLE COLLECTION

The amount and method of collection of material for leaf area determination will depend on the objective of the experiment. However, some general guidelines are worth noting.

- Foliage for which leaf area is to be determined should be collected and placed in a sealed container and stored at temperatures between 1.5-4.5°C if storage is required.
- Foliage should not be stored more than 4 days before leaf area determinations are made or leaves will begin to deteriorate.
- The containers in which the foliage samples are stored should be clearly marked with all pertinent treatment identification information.

6.3.1.2. SAMPLE COLLECTION AND STEPS FOR DESTRUCTIVELY DETERMINING WHOLE SEEDLING LEAF AREA

1. Identify the number of different morphological classes of needles to be sampled (e.g., primary vs. secondary, first vs. second flush).
2. Collect all foliage and identify by morphology class.
3. Select a 10% (fresh weight basis) subsample at random from each morphology class.
4. Determine the leaf area of the subsample by procedures outlined in Section 6.3.2.
5. Determine the oven dry weight (70°C) of the subsamples.
6. Determine oven dry weight (70°C) of all samples.
7. Use subsample measures of leaf area and oven dry weight to determine specific leaf area (cm^2/g).
8. Multiply total dry weight of each morphological class by the specific leaf area determined for each class to determine the total leaf area of each seedling.

6.3.2. MEASUREMENT

6.3.2.1. MATHEMATICAL METHOD (DISPLACEMENT METHOD)

A procedure for estimating leaf area by using a mathematical relationship between a leaf characteristic and total surface area has been developed by Johnson (1984). As he describes in his publication on this technique, "success lies in the ability to mathematically describe needle geometry and to reproducibly measure needle volume to 0.01 ml using Archimedes principle." Johnson assumes that

"A pine needle in cross-section approximates a sector with a radius, r, and an angle, θ. The angle, θ, is a function of the number of needles per fascicle (θ = 360/n).

The volume of a sector is described by the following equation:

$$V = \left[\frac{\pi r^2}{n}\right] \ell \qquad (1)$$

where V is volume (cm^3), r is the radius of the sector (cm), n is the number of needles per fascicle, and ℓ is the length of the needle (cm). The surface area of a sector is described by

$$A = \left[2r + \frac{2\pi r}{n}\right] \ell \qquad (2)$$

To reduce the number of measurements, equations (1) and (2) are first solved for r:

$$r = \sqrt{\frac{Vn}{\pi \ell}} \qquad (3)$$

$$r = \frac{A}{2\ell + \frac{2\pi\ell}{n}} \qquad (4)$$

and then combined, solving for A, equation (5):

$$A = 2\ell \left[1 + \frac{\pi}{n}\right] \sqrt{\frac{Vn}{\pi \ell}} \qquad (5)$$

This is the final form of the total surface area equation where A is the total surface area (cm^2), V is the displaced volume of the needle sample (cm^3), n is the number of needles per fascicle, and ℓ is the comulative needle length of the needle in the sample (cm)."

To determine the volume and radius, the following procedures were used.

"As the fascicles were removed from a branch, they were cut at the basal sheath and the entire needle sample of 20 to 30 needles was wrapped with a thin wire. The sample was loosely wrapped with wire to avoid the trapping of air bubbles around the needles, and if air bubbles were still a problem, a surfactant can be added to the water. The volume of the sample was then determined to the nearest 0.01 g by volume

displacement on a Mettler A30 balance. The displaced volume of the wire was measured and subtracted from the total volume. Once the volume was measured, each needle in a sample was individually measured for its length to the nearest millimeter and for radius to the nearest 0.005 cm with a caliper.

...Two problems were encountered in attempting to measure a representative mean radius: (1) needle taper varied with the number of needles per fascicle, and (2) measurement error was considerable due to needle compression by the caliper. Needle taper was determined by measuring the radius at 2 mm intervals along the length of a number of needles from each species.... Using a caliper to measure needle radius proved to be less than satisfactory due to the resolution of the caliper (0.005 cm) and associated measurement errors resulting from needle compression in the caliper. An error of 0.001 cm in radius was calculated to result in a 3 percent change in surface area.

...To measure a large number of needles, the fastest method was to measure individual needle lengths in intact fascicles. Length was measured from the top of the basal sheath to the tip (rounding down to the nearest millimeter) and then the needles were cut at the basal sheath prior to measuring volume. These individual needle lengths were summed to give a cumulative needle length for each sample." (Johnson, 1984).

6.3.2.2. PLANIMETRIC METHODS

Procedures for operating the LI-3100 follow:

"Connect the supplied power cord to the power input connector at the rear of the instrument. A grounded three prong wall connector is required for electrical service. Move the 'on-off' switch to 'on.' Press the "lamp start" firmly and release after holding for approximately two seconds. If the fluorescent tube does not illuminate, repeat the procedure. Press the reset button to clear the display.... The display will rapidly accumulate numbers when the 'on-off' switch is initially placed in the 'on' position. This accumulation will continue until the fluorescent tube is activated. If numbers continue to accumulate, then the calibration screw should be adjusted counterclockwise until the displayed numbers remain constant. Accurate or larger than actual calibration disk measurements are normal at this calibration screw adjustment. Data accumulation may continue to occur if the fluorescent tube output is not sufficient (as with initial starting in extremely cold conditions). Excessively low line voltage is also a cause for continued rapid spurious counting." (LI-COR, 1979)

In operating the Delta-T leaf area meter,

"... a TV camera views the object to be measured, which is illuminated to contrast with its background. The Area Meter Box sums the periods during which the line by line camera scan is traversing an object. This sum is a measure of the area and can easily be adjusted for calibration using an object of known area. The output from the TV camera is displayed on a monitor, together with a superimposed image of the actual measured area. A digital display on the monitor screen shows the area of the object in view, the number of measurements made, and the total area.

An object will be detected and measured if the Area Meter Box can identify it as being light or dark in relation to the background. The Threshold Control sets the grey-level at which an object will be detected. The effect of adjusting the Threshold is seen on screen as a progressive blacking-in of the object. When the whole shape is black the measured area can be read off from the display. Once set for a particular type of object the level does not need readjustment." (Delta-T Devices, Ltd.)

Both leaf area meters are appropriate for broadleaf foliage where surface area is primarily a function of leaf length and breadth. Suitability for use on conifer foliage lessens because needle breadth approaches needle thickness (Drew and Running, 1975). A regression or conversion factor must be employed to relate projected surface area to total surface area (Carlson and Johnstone, 1979) unless the needles are flat.

6.3.2.3. GENERAL STEPS TO FOLLOW IN DETERMINING LEAF AREA BY THE PLANIMETRIC METHOD

1. Let machine warm up 3-5 minutes.

2. Check the calibration (Section 6.5) before beginning a measurement session. Use a calibration plate that is in the mid-range of the area measurements to be made and with a similar configuration as the foliage being measured. Take five measures so both accuracy and precision can be determined.

3. For multi-needle conifer - clip the needles off just above the fascicle bundle sheath.

4. Place the sample as perpendicular to the sensing element as possible. This is only important for narrow leaf conifers.

5. Make sure foliage does not overlap.

6. After every 50 sample measurements, recheck the accuracy and precision with a plate as described above for known area. If the

reading of the calibration plate exceeds +5%, the belt should be cleaned and adjusted if needed. If this does not reduce the error to less than 5%, corrective action must be taken before measurements proceed. A record of calibration checks and average deviation of the readings from the known area plots must be maintained.

6.4. PREVENTIVE MAINTENANCE

Maintenance for the LI-3100 involves cleaning of the belts, mirror, and occasionally the camera lens. Clean the belts with water and a cloth or absorbent paper. A detergent may be used for persistent contamination but do not allow detergent to fall on the mirrors. Any scrubbing of the mirrors to remove detergent spots may damage the mirror surface. Access to the lower belt is facilitated by momentarily activating the "on-off" switch to present surface near the sample tray. The inner surfaces are cleaned by reaching into the access ports in the front plate. Loosen the belts to facilitate access to the pulley surfaces.

If the mirror or camera lens must be cleaned, use the "blow brush" provided with the instrument. If persistent dirt remains on the mirror, use water and a soft absorbent paper such as lens paper.

Cleaning within the camera is not a frequent requirement but when necessary, follow these steps. Remove the lens. Loosen the screws on the outer camera pressure plate and lift the camera from the rails to more easily inspect the interior. The camera remains connected so do not apply tension to the connection. The rectangular sensitive device (RETICON) is visible at the interior rear of the camera housing. Any speck of dirt on this sensor will cause spurious counting. Use the "blow brush" provided to remove dust. Do not place a moist cloth within the camera. The adhesive dust retaining surface surrounding the RETICON and printed circuit board would be damaged.

Maintenance for the Delta-T is similar.

6.5. CALIBRATION PROCEDURES

A warming period of 1-3 minutes may produce a calibration change of approximately 1% from that obtained at the initial starting. This is a fluorescent tube output response. Calibration should be performed 3-5 minutes after tube illumination.

Calibration is performed on both leaf area meters by placing a calibration disk between the sample guides on the sample tray. Reset the display and slide the disk onto the lower transparent belt and allow the disk to travel through the instrument. Turn the "CAL" screw clockwise to increase the displayed sample area. If the displayed area is too large, adjust the "CAL" screw counterclockwise. When proper calibration has been achieved, subsequent measurements of the calibration disk should result in an error less than 5% of the actual area.

6.6. CALCULATION/UNITS

The surface area of foliage is to be given in cm^2. For conifers, the surface area is to be total surface area. This means all measures of projected leaf area must be converted to total leaf area. For hardwoods, the area of the foliage will be expressed as a single sided area.

6.7. ERROR ALLOWANCE AND DATA QUALITY

With optical planimeters, a dimensional correction factor must be developed for each species measured. The precision of the LI-3100 was determined at the 97% level with irregular-shaped complex objects. Most applications will result in less error. A table of accuracy is shown below (LI-COR, 1984).

Resolution	10 cm^2	5 cm^2	1 cm^2	0.5 cm^2	0.25 cm^2
1 mm^2	± 1%	± 2%	± 5%	± 7%	--
0.1 mm^2	± 0.5%	± 1%	± 1%	± 1.5%	± 4%

For the Delta-T, resolution of 1/300th of the scanned width and height is achieved. It ranges from 1 mm for large areas (360 x 260 mms), to 0.02 mm for small areas (35 x 26 mms). Accuracy ranges from 96-99%, depending on factors such as size, shape, and contrast. The operator can maximize accuracy and resolution by setting the camera to the lowest height permitted by the sample dimensions, (so that the whole of the object remains within the scanned area). Other significant factors affecting accuracy are the amount of contrast between object and background, and whether the object has features close to the resolution limit.

6.7.1. DATA QUALITY OBJECTIVE

Variable	Reporting Units	Repeated Measurement Error at Lower Limit	Repeated Measurement Error at Upper Limit	Measurement Accuracy Tolerance
Leaf Area	0.01 cm^2	± 2%	± 5%	5%

6.8. REFERENCES

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7. STANDARD OPERATING PROCEDURE FOR SEEDLING GROWTH MEASUREMENTS

7.1. SCOPE AND PURPOSE

Measures of seedling growth will be the major response variables used to evaluate the impact of the assigned treatments to be tested under the Forest Response Program. In many cases not only is the impact on total productivity important, but also shifts in carbon allocation or growth form that may result from these treatments are important. This SOP outlines the procedures necessary to determine the weight change of the foliage, stem, and root components for two type studies: (1) growth chamber studies and (2) open-top chamber studies.

7.2. MATERIALS AND SUPPLIES

- Meter stick - graduated in cm
- Calipers
- Analytical balance

7.3. PROCEDURES

7.3.1. GROWTH CHAMBER STUDIES

7.3.1.1. INITIAL SEEDLING MEASURES

1. Select 20 seedlings/family at random from the population that is available for use in the exposure study.
2. Determine height from the root collar (original ground line) to the tip of the bud with a meter stick.
3. Determine root collar diameter with a caliper at 1 cm above the original root collar.
4. Separate each seedling into foliage, stem, and root components.
5. Determine oven dry weight (70°C) of each component.
6. Develop equations relating seedling height and diameter to component dry weights.

7.3.1.2. ESTIMATING INITIAL WEIGHTS OF STUDY SEEDLINGS USING RELATIONSHIPS DERIVED FROM SUBSAMPLE

1. Measure height and root collar diameter (as in 7.3.1.1) for all seedlings going into the growth chamber.
2. Use the equations derived in 7.3.1.1 to estimate initial seedling weight by components (foliage, stem, and root).

3. Mark each container so subsequent measures of diameter and height are made from the same side.

4. Note: Plant seedlings so that root collar diameter can be measured at the same location as in 7.3.1.1.

7.3.1.3. ESTIMATING INTERIM AND FINAL WEIGHTS

1. Measure heights and diameters on a monthly basis using a meter stick and calipers (optional).

2. At the final measurement date repeat all the initial steps outlined in 7.3.1.1.

7.3.2. OPEN-TOP CHAMBER STUDIES

7.3.2.1. INITIAL SEEDLING MEASURES

1. Select 20 seedlings/family at random from the population that is available for use in the open top chamber study.

2. Determine height from the root collar (original ground line) to the tip of the bud with a meter stick.

3. Determine root collar diameter with a caliper at 1 cm above the original root collar.

4. Separate each seedling into foliage, stem, and root components.

5. Determine oven dry weight (70°C) of each component.

6. Develop equations relating seedling height and diameter to component dry weights.

7.3.2.2. INITIAL CHAMBER SEEDLING MEASUREMENTS

° Just prior to beginning treatment exposures, height and root collar diameter of all seedlings should be measured.

7.3.2.3. END OF YEAR 1 MEASUREMENTS

1. Select 10 trees/family/treatment for destructive harvesting.

2. Divide the growth measurement trees into stems and foliage. Note: No root biomass measures are required.

3. Randomly select a subsample of foliage from each family and determine the specific leaf area (cm^2/g) as outlined in the SOP for Measurement of Seedling Leaf Area (Section 6.3.2.).

4. Determine oven dry weight of the stem and foliage components.

5. Determine the relationship between stem and leaf biomass and measured diameter and height.

6. Estimate total leaf area from specific leaf area measurement and leaf biomass measures.

7. Measure height and diameter of all seedlings remaining in the chamber.

8. Estimate stem and leaf biomass of the seedlings that are to remain in the study for year 2.

7.3.2.4. END OF EXPERIMENT GROWTH MEASURES

1. Measure height and diameter of all seedlings.

2. Clip all seedlings at the root collar.

3. Separate into foliage and stems.

4. Select a subsample of foliage and determine specified leaf area for each family. See SOP for Measurement of Seedling Leaf Area (Section 6.3.2.).

5. No root biomass estimates are required.

7.4. PREVENTIVE MAINTENANCE

° Keep caliper clean and oiled.

° Keep meter sticks in a container so they do not get damaged and become difficult to read.

7.5. CALIBRATION PROCEDURES

1. At the beginning and end of a measurement period, the caliper should be checked against a calibration gauge of known width.

2. After each reading, the caliper should be checked to ensure it reads zero when the jaws are fully closed. If it does not, clean the jaws if they have resin on them and adjust the caliper to read zero.

3. Determine caliper accuracy and precision by measuring the calibration gauges 5 times at the beginning and end of a measurement day.

4. Analytical balances should be calibrated at the beginning and end of each measurement session using a set of standard weights which cover the range of measurements to be made. Readings should be within 1% of the known weight readings. Analytical balances should be spot checked using a single calibration weight that is

near the mid point range of the samples being measured. If a spot check deviates more than 1% from the known value, the analytical balance should be checked for cleanness. If cleaning does not help, the instrument should not be used until it can be recalibrated.

7.6. CALCULATIONS/UNITS

- Relative growth rate calculations should follow the procedures described by Evans (1972).
- Seedling biomass estimates from diameter and height measures can be estimated using the procedures described by several authors (see Section 7.8. References).

7.6.1. UNITS

Root collar diameter.....mm-nearest tenth mm

Height.....cm-nearest half cm

Weight.....mg-nearest mg

Specific leaf weight.....$cm^2\ mg^{-1}$

7.6.2. RECORDING FORMAT

Date.....0-365

Time.....Standard Military

Treatment.....Number

Family.....Numeric code

Tree.....Number

Height.....cm-nearest half cm

Diameter.....mm-nearest tenth mm

Weight-foliage.....mg-nearest mg

Weight-shoot.....mg-nearest mg

Weight-root.....mg-nearest mg

7.7. ERROR ALLOWANCEF AND DATA QUALITY

Errors in growth measurements can result from errors in the measurement of height, diameter, or weight. Height can be measured to the

nearest 0.5 cm. Diameter can be measured to the nearest .01 mm. Due to variability in stem dimension, diameter estimates made with a single stem measurement will be only accurate to the nearest 0.1 mm.

Weight measurements will be made to the nearest 1% of actual weight.

7.7.1. DATA QUALITY OBJECTIVES

Variable	Reporting Units	Repeated Measurement Error at Lower Limit	Repeated Measurement Error at Upper Limit	Measurement Accuracy Tolerance
Seedling Height	0.5 cm	± 2%	± 2%	2%
Sapling Height	0.1 m	± 5%	± 5%	5%
Diameter	mm	± 5%	± 5%	5%
Plant Dry Weight	mg	± 1%	± 1%	2%
Root Weight	mg	± 1%	± 1%	2%
Stem Weight	mg	± 1%	± 1%	2%
Needle Weight	mg	± 1%	± 1%	2%

7.8. REFERENCES

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Hatchell, G.E., C.R. Berry, and H.D. Muse. 1985. Non-destructive indices related to aboveground biomass of young loblolly and sandpines on ectomycorrhizal and fertilizer plots. For. Sci. 31:419-427.

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8. STANDARD OPERATING PROCEDURE FOR DETERMINATION OF FOLIAR INJURY TO SEEDLINGS AND SAPLINGS

8.1. SCOPE AND PURPOSE

The assessment and description of foliar injury under controlled conditions such as growth chamber and open-top chamber studies can be useful in (1) determining how much functional foliage has been reduced by exposure to pollutants and (2) developing guides for identifying observed field damage. The proposed system will attempt to describe the extent of damage as well as the cause of damage.

8.2. MATERIALS AND SUPPLIES

Illustrations of foliage having damage in 10% area intervals for the species being studied should be developed to serve as guides for estimating foliage damage.

8.3. PROCEDURES

8.3.1. SYSTEM FOR FOLIAGE DAMAGE ESTIMATES

The following system should be used to make estimates of visible foliage damage.

Vigor Class	Extent of Damage	Types of Damage
0	None	
1	≤10%	
2	>10% ≤20%	
3	>20% ≤30%	
4	>30% ≤40%	
5	>40% ≤50%	
6	>50% ≤60%	
7	>60% ≤70%	
8	>70% ≤80%	
9	>80% ≤90%	
10	>90%	

Types of damage could be chlorosis, necrosis, desiccation, or insects.

8.3.2. STEPS IN DETERMINING FOLIAR DAMAGE LEVEL

1. Use random procedures to select foliage to be assessed for damage.

2. Compare sample foliage with a set of standards having a known percent of area randomly shaded.

3. Visually estimate the extent of foliar damage using the standard guide.

4. Code the type or types of damage that are visible.

5. Note: A designated person should make all the estimates if possible. The person should coordinate and train all other individuals estimating visual injury for that species.

8.4. PREVENTIVE MAINTENANCE

N/A

8.5. CALIBRATION PROCEDURES

N/A

8.6. CALCULATIONS/UNITS

Damage will be expressed in percent. The amount of damaged area for the whole seedling can be estimated from the randomly selected damage assessed foliage.

8.7. ERROR ALLOWANCE AND DATA QUALITY

It is unlikely that the total damage area can be estimated more accurately than +20% except when damage approaches 100% or zero.

8.8. REFERENCES

N/A

9. STANDARD OPERATING PROCEDURE FOR MYCORRHIZAL ASSESSMENTS

9.1. SCOPE AND PURPOSE

This SOP will outline the procedures available for quantifying ectomycorrhizae on seedling roots for seedlings grown in containers for up to one year. Due to sampling complications where multiple families are grown in open-top chambers for 2-3 years, no procedures are being recommended in this SOP for conducting mycorrhizal assessments in open-top chamber studies.

9.2. MATERIALS AND SUPPLIES

- Water source for removing potting media
- Microscope
- Soil sieves or screens
- Ruler
- Analytical balance
- Defloculation agents
- Plant material: The entire root system should comprise the sample for seedlings grown in containers.

9.3. PROCEDURES

9.3.1. SAMPLE PREPARATION

Sample collection and preparation should follow the procedures outlined by Grand and Harvey (1982). The number of plants that has to be sampled cannot be specified for all studies. It will depend on the variability in mycorrhizal infection in the study being conducted. In studies that are inoculated in containers, usually a minimum of ten plants per replication and 4-5 replications are required to show significant differences at the .95 significance level.

9.3.2. MEASUREMENT - COUNTS

The method used to quantify the level of mycorrhizal infection will depend on the study objectives, the size of the seedling, and the number

of seedlings that have to be assessed. The methods that have been used are:

- Direct counts of the entire root system (this is suitable for small numbers of seedlings),
- Direct counts of randomly selected roots (this procedure permits assessment of more seedlings), and
- Counts of ectomycorrhizal tips (this procedure counts tips of live and/or dead mycorrhizae. It should be the preferred method if the study objective is to relate mycorrhizal activity to nutrient cycling, turnover, etc).

These methods have been described in detail by Grand and Harvey (1982).

Precision estimates can be made by having each person that is in charge of making assessments count mycorrhizae on a set of ten, fifteen cm root segments and repeat this five times.

9.4. PREVENTIVE MAINTENANCE

N/A

9.5. CALIBRATION PROCEDURES

There are no instruments to be calibrated. In nutrient cycling studies, weight may have to be determined. Precision and accuracy and calibration requirements outlined in Appendix B are applicable.

9.6. CALCULATION/UNITS

Results may be presented as

- number of ectomycorrhizae per seedling.
- number of ectomycorrhizae/unit length of root.
- percent of short roots with ectomycorrhizae.
- weight of ectomycorrhizal tips/unit area or weight of soil.
- weight of ectomycorrhizal tips/unit volume of soil.

9.7. ERROR ALLOWANCE AND DATA QUALITY

In container studies involving seedlings, the major sources of errors will be:

- loss of fine roots in extracting the roots for mycorrhizae counts

for studies designed to assess mycorrhizae, using a 3:1 vermiculite:peat potting mix will facilitate root extraction), and

- counting all forked short roots as mycorrhizal roots (as much as 15% of forked roots may not be mycorrhizal).

To minimize errors in mycorrhizal counts, it will be necessary that certain people be assigned the task of making all mycorrhizal counts. These people should attend a short course which covers how to

- recognize mycorrhizal roots,
- collect root samples,
- store samples,
- count mycorrhizal roots, and
- quantify mycorrhizal tips.

9.8. REFERENCES

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APPENDICES

APPENDIX A

List of Attendees
Exposure Systems and Physiological
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QA Methods Manual Development
Raleigh, NC
11-12 March 1986

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Table 1. Table of measurements, SOP references and accuracy & precision levels required for measures of net carbon exchange (NCE), water vapor exchange, xylem pressure potential and the required associated environment & plant variables.

Measurement/Method	Standard Operation Procedure Citations	% Precision	% Accuracy	Additional Operation Instruction/Requirements	Calib Frequency	Spot Check Frequency	Cautions: Comments
Net Carbon Exchange (NCE)	LI 6000 - Manual			• Boundary layer resistance ≤ .25 s/cm			
	ADC-LCA2 - Manual			• Cuvette light transmittance 85% of a new chamber.			
	Sestak, Catsky & Jarvis - Plant Photosynthesis Production Manual of Methods			• Leaf temperature elevation ≤ 2°C due to enclosure of foliage within the cuvette.			
				• Draw down of CO_2 concentration should be ≤ 15 ppm for a closed system measurement of NCE at ambient CO_2 levels.			
• CO_2 Analyzer & Chambers		< 1% variation	± 5 ppm		Span - Daily & Zero - Daily	Zero - hourly	
• Leaf & Air Temperate	Thermistors, thermocouples	± 1.0°C	± 1.0°C	• Conifer leaf temperature to be taken as air temperature taken with shielded thermocouple mounted in the chamber.	3 months	Compare chamber & unit thermistors daily.	• Avoid chamber thermistor being exposed to direct sunlight. • Avoid long measurements which lead to high foliage temperature buildups under high temperature & high radiation loading. Temperature should be held with 2°C of ambient.
• Photosynthetically Active Radiation	Quantum sensor	± 2%	± 5%	• All measurements to be taken with sensor held level.	Annual	Note deviations from full sunlight. Full sunlight will be close to 2000 µmoles/m²/s. If deviation from full sunlight is greater than 400 µmoles/m²/s on a clear day at solar noon during the summer period. Check the sensor against another sensor that has a more recent calibration.	
• Relative humidity	Thin layer capacitor type sensor	± 2%	± 5%		Weekly		When operating under humidities > 90% with strong oxidants in the atmosphere, calibration should be checked twice weekly until it is established whether rapid deterioration of the sensor is occurring. If water condenses on sensor, let dry & recalibrate before reusing.

Table 1. Continued

Leaf Area	*LI 3000 *LI 3100 - Manual Delta-T - Manual	± 5%	± 5% (Projected Area)		A calibration is to be made at the beginning and end of each measurement session with a calibration plate that has a similar area & configuration of the individual tissue sample being measured.	Recheck calibration after every 50 samples.	No matter which projected area method is selected for use with conifers that do not have flat needles, it will be necessary to develop a calibration curve relating projected area to total surface area. When the study involves both primary & secondary needles, separate calibration curves will be required for each needle type.
	*Volume Displacement Methods	± 5%	± 5% (Total Surface Area)		Non-required		
	Volume Displacement • Jon D. Johnson (1984) - Appendix A Rapid Technique for Estimating Total Surface Area of Pine Needles • Shelton, M.G. & G.L. Switzer (1984) - Appendix • McLaughlin, S.B. & H.A.I. Madgaick (1968) • Gurumurti, K. & V.K.S. Rirastara (1982)						
Leaf Weight	Weight Measurement Section NCLAN Q/A Plan for Biological Measurements	1%	± 1.5%		Calibrate at the beginning & end of each weighing session. If weighing session is greater than 4 hours, check every 4 hours.	Spot check a mid point standard weight every 30 measurements.	
Stomatal Conductance Stomatal Resistance	LI-1600 - Manual Delta-T - Manual			Same as for NCE measurements described above, except for references to CO_2 conditions.	Factory Calib annually	Same as for NCE above.	
Xylem pressure potential	PMS & Soil Moisture Test Owner's Manual	± 0.1 MPa Establish precision estimate across the range of xylem pressure potentials that are experienced in the study.	No check		None required	Check that pressure needle goes to zero after each XP determination.	

LI-6000 Portable Photosynthesis System

Service included with 6000CAL Factory Calibration

Calibrations

1. Humidity Sensor*
2. CO2 Analyzer relinearization* (7 gas concentrations at 3 temperatures; 18 linearization points are derived from a computer program)
3. Flow meter relinearization (7 different flow rates at 3 temperatures)
4. Chamber temperature
5. Leaf temperature
6. Quantum sensor

* customer is informed of what the reading was when it was received and what the new values are. This shows the amount of calibration drift that occurred. Calibration certificate sent with each instrument.

Tests

1. Check all 6000B Rechargeable Batteries that are returned:
   a. Charge overnight
   b. Timed discharge at 1 amp
   c. Evaluate results
   d. Charge overnight
   e. Force charge for a period of time, dependent on step 3 evaluation
   f. Timed discharge again
   g. Repeat until 2 amp-hour reached or battery is not able to be revived (generally 2 force charges is the maximum needed to determine this).
   h. Customer is informed of battery results and whether replacements are needed.
2. Test pump (air flow rate).
3. Check and fix any air leaks in the system (inform customer of leaks found).
4. Check accuracy and repeatability of CO2 analyzer and flow meter.
5. Check noise levels of CO2 analyzer and flow meter.
6. I/O check (dump data to terminal or printer).
7. Test A/D, multiplexer accuracy/linearity.
8. Check chamber fan current drain; replace fan if excessive (fan not included).

General Maintenance

1. Replace Ni-Cad back-up batteries for RAM board if over one year old.
2. Replace air filters in LI-6050.
3. Check for loose nuts and screws.
4. Replace chamber pads as needed.
5. Clean chambers and instruments as needed.
6. Replace hoses as needed.
7. Connectors: Replace O-rings and grease as needed.
8. Install any upgrades that are included as no-cost upgrades.
9. Replace soda lime with new soda lime.
10. Replace 6000DP filter disk on desiccant tube as needed.

APPENDIX D

COMPUTER CODES AND REPORTING SYMBOLS FOR CERTAIN MEASURED VARIABLES

Variable Name	Code	Reporting Symbol
Leaf Conductance	LCOND	g_{lj} [1/]
Leaf Resistance	LRES	r_{lj}
Canopy Conductance	CCOND	g_{cj}
Transpiration	TRAN	E_t
Photosynthetically Active Radiation	PAR	PAR
Air Temperature	ATEMP	T_a
Soil Temperature	STEMP	T_s
Relative Humidity	RHUM	RH
Leaf Temperature	LTEMP	T_l
Vapor Pressure Deficit	VPD	VPD
Vapor Pressure Gradient	VPG	VPG
Soil Water Potential	SH2O	Ψ_s
Soil Water Content	SWATR	θ
Xylem Pressure Potential	XPP	XPP
Specific Leaf Area	SLA	SLA
Leaf Surface Area	LAREA	A_l
Leaf Area Index	LAI	LAI
Apparent Net Photosynthesis	NPS	A_n
Dark Respiration	DRESP	A_r
Ambient Carbon Dioxide	CO2A	c_a
Internal Carbon Dioxide	CO2I	c_i
Photorespiration	PRESP	A_{pr}

1/ lj refers to species -- ----nts or molecule eg. G_{lCO_2} = leaf conductance to CO_2

LI-1600 STEADY STATE POROMETER
1600CAL FACTORY CALIBRATION DESCRIPTION

Calibrations

1. Relative Humidity* (standard range 25-75%)
2. Mass Flow Meter*
3. Cuvette Temperature
4. Leaf Temperature
5. Quantum Sensor

* calibration certificate sent with each instrument includes readings taken before and after calibration, indicating the amount of drift that has occurred.

Tests

1. Test pump (air flow rate)
2. Check for and repair any air leaks in the system. (Customer informed of leaks found.)
3. Check cassette and RS-232 interface operation.
4. Check HOLD switch for proper operation
5. Check "HUM SET" switch for proper operation.
6. Check power supplies for proper voltages. Calibrate if necessary.
7. Check flow controller for proper operation. Calibrate if necessary.

General Maintenance

1. Check for loose nuts and screws.
2. Clean instrument as needed.
3. Replace aperture pads as needed.
4. Replace hoses as needed.
5. Connectors: Replace and grease "O-Rings" on air line connectors as needed.
6. Check ribbon cables and connectors.
7. Visually check wiring connections.
8. Perform any routine upgrades.
9. Replace used dessicant with fresh dessicant.
10. Re-form leaf temperature thermocouple.
11. Check both fans for proper operation.
12. Recharge battery.

Replacement Parts Included

1. Urethane tubing for dessicant pack (2 pieces 2 1/2" long)
2. Spare aperture pads (3)
3. Desiccant

Price: U.S. $150 plus shipping. Other repairs needed are invoiced in addition to the 1600CAL price.

LI-COR, inc. / LI-COR, Ltd.
Box 4425 / Lincoln, Nebraska 68504 USA
Phone (402) 467-3576 / TWX: 910-621-8116

APPENDIX F

NATIONAL CROP LOSS ASSESSMENT NETWORK

QUALITY ASSURANCE PLAN

FOR BIOLOGICAL MEASUREMENTS

Approved: _____ Date: 9/24/85
NCLAN Biological Quality Assurance Leader

Approved: _____ Date: 10/7/85
NCLAN, RMC, Chairman

Approved: _____ Date: 9/27/85
EPA Project Officer

Approved: _____ Date: 9/30/85
APEB, Chief

Approved: _____ Date: _____
CERL Quality Assurance Officer

All responsible personnel listed in the NCLAN Project will receive this quality assurance plan for biological measurements plus any necessary revisions.

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LIST OF FIGURES

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1.0 Introduction

The objective of this quality assurance plan is to assure that biological data collected for the National Crop Loss Assessment Network (NCLAN) is of known quality, accuracy, and legally defensible. NCLAN objectives, experimental designs, use of data, instrumentation QA plan, personnel, and locations are found in the NCLAN Project Plans. This document specifies quality assurance procedures required for biological measurements associated with NCLAN research.

2.0 Scope

Biological measurements made in NCLAN studies may include plant weights, crop quality, soil moisture, and physiological measurements such as chlorophyll content, leaf water potential, leaf conductance (resistance) and/or photosynthesis. The procedures outlined here are used to monitor these variables and assure consistent biological quality assurance at each NCLAN location.

Although the NCLAN program is designed to conduct research using a common basic research protocol, because of regional, experimental, and/or crop differences, the same measurements are not always made across sites or years. Since NCLAN is a dynamic air pollution-crop research program, personnel frequently develop and test new techniques, methods, and apparatus for making biological measurements. When such procedures and/or apparatus are developed, approved plans for assuring data quality will be added to this document before procedures or equipment are used in the research.

3.0 QA Personnel and Responsibilities

1. NCLAN Research Management Committee (RMC)

The RMC manages all work conducted in NCLAN studies. NCLAN quality assurance plans must be approved by this committee:

Walter W. Heck, Chairman, Agricultural Research Service, U.S. Department of Agriculture, Raleigh, North Carolina

O. C. Taylor, Associate Chairman, University of California, Riverside

Richard M. Adams, Oregon State University, Corvallis

Lance W. Kress, Argonne National Laboratory, Illinois

David T. Tingey, Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, Oregon

Leonard H. Weinstein, Boyce Thompson Institute, Ithaca, New York

2. NCLAN Biological Quality Assurance Leader (NCLAN-BQAL)

The BQAL for all NCLAN locations is located at the Corvallis EPA Laboratory. Responsibilities include (a) approving and monitoring all QA plans; (b) compiling QA forms and preparing annual evaluation reports. The NCLAN-BQAL (or designee) conducts at least one site audit each year, contingent upon availability of travel funds.

3. Site Leader

The Senior Scientist at each location is the BQAL unless otherwise designated. Responsibilities include (a) ensuring that all QA plans are followed at that site; (b) preparing and obtaining approval for detailed QA plans when appropriate procedures are not

included in the NCLAN QA document; (c) maintaining current samples of approved verification, data sheets, and QA forms; (d) submitting QA forms to the NCLAN BQAL by January 1; (e) reviewing notebooks for completeness, calibration data, and preventive maintenance documentation.

4.0 QA Objectives and Procedures for Biological Measurements

A summary of biological variables to be measured, methods, equipment, referenced standard operating procedures, minimum precision and accuracy levels is shown in Table 1 in the Appendix.

For verification and tracking purposes, all samples and measurements are coded so that the researcher can determine:

a. study number or species

b. harvest date and/or number

c. block or replicate number

d. treatment number or name

e. sample number

f. name of person(s) taking sample or measurement

1. Weight Measurements

The economically important portion of each crop is harvested at maturity or, in case of fresh produce, when it is normally marketed. Other plant parts also may be harvested for experimental purposes. All crop material grown in the central 8-foot diameter of an open-top chamber is normally harvested. Crop material from each quadrant is harvested, yielding 4 samples per plot. Special studies may require samples from smaller areas and in these experiments, the sampling will be conducted according to the recommendations of the NCLAN statistician.

The exact weight of samples is not required for NCLAN studies. It is essential that all samples from a harvest or group be weighed in the same manner. Use of procedures outlined in this section

ensure that all samples are weighed within 1.5% of their true weight.

Each NCLAN location maintains a set of standard weights verified annually to 0.5% of another set of known standard weights. Figure 1 illustrates a typical weight verification form. Scales or balances are calibrated at the beginning and end of each weighing period. For weighing sessions over 4 hours, a mid-session calibration check is conducted. Weights used for calibration will be representative of the mid-range of samples to be weighed. To begin or continue weighing each day, calibration checks must be within 1% of the known value. Weighed samples are kept separate until validated by a second calibration. If the mid-session or end-of-day calibration is not within 1% of the known weight, all samples weighed since the last valid calibration will be considered void. They will be weighed again and considered validated when a valid before- and after-calibration check is conducted as stated above. Figure 2 illustrates a typical calibration verification form.

When a calibration check deviates more than 1% of the known value, the operator(s) first consult the operating manual to identify possible problem areas. If unable to obtain a valid calibration, the operator(s) notify the BQAL for the site to initiate corrective action. This may indicate the need for a maintenance check by a qualified equipment representative.

NCLAN Quality Assurance Weight Verification Check

NCLAN Location:

Operator(s):

Brand Name and Serial #

$$\% \text{ Difference} = \frac{\text{Working Wt} - \text{Standard Wt.}}{\text{Standard Wt.}} \times 100$$

	Weight Number								
	1	2	3	4	5	6	7	8	Comments
Date									
Working									
Standard									
% Diff.									
Date									
Working									
Standard									
% Diff.									
Date									
Working									
Standard									
% Diff.									
Date									
Working									
Standard									
% Diff.									

Figure 1

NCLAN Quality Assurance Calibration Checks for Weight Measurements

NCLAN Location:

Study Name:

Operator(s):

Brand Name and Serial #:

Description of Samples:

$$\% \text{ Difference} = \frac{\text{Measured Wt. - Working Wt.}}{\text{Working Wt.}} \times 100$$

Date	Start-Up Check			Mid-Session Check			End of Day Check			Comments
	Measured	Working	% Diff	Measured	Working	% Diff.	Measured	Working	% Diff.	

Figure 2

2. Soil Moisture Measurements

Soil moisture measurements are made using the neutron probe and/or tensiometers depending on equipment availability and/or site soil characteristics.

In NCLAN studies, tensiometers are used to measure soil moisture potential in atmospheres. Because of the nature of tensiometers, it is only possible to conduct before- and after-study calibration checks. This is done by placing all probes at the same depth in a soil of uniform moisture or in a container of water. Readings are recorded by probe number (see form in Figure 3). A soil sample may be taken at the same depth as the tensiometers to obtain a direct measure of soil moisture (gravimetric) for comparison.

A probe that does not read within 10% of the median level probe will not be used in the study. Probes reading zero (defective) in the pre-study check will not be used to calculate the median level probe. Probes deviating from the 10% standard at the end-of-study check will be recorded as suspect of invalid readings for the entire study. Each site maintains a 10% extra supply of probes that meet the 10% uniformity standard. These will replace defective probes during the study If a probe is suspected of erroneous readings, the operator first checks to see that the manufacturer's recommendations are being followed. If the probe is still suspect, the operator notifies the site BQAL who will verify the need to pull the probe, check it against the extras for verification, and if necessary, replace it. See Table 1 for additional QA information.

NCLAN Quality Assurance Calibration Checks for Soil Moisture Tensiometers

NCLAN Location:

Study Number:

Study Name:

Person(s) Calibrating:

Date Began Calibration: Date Ended Calibration:

$$\% \text{ Difference} = \frac{\text{Measured} - \text{Median}}{\text{Median}} \times 100$$

Probe No.	Measured	Median	# Diff.

Probe No.	Measured	Median	# Diff.

Comments: _____

_____

_____

_____

_____

Figure 3

The neutron probe is also used to measure percent soil moisture. See Table 1 for QA information for this method.

3. Plant Health

Plant health is determined by daily visual examination of treatment plots. Incidence of disease, pathogen, insect, or nutrient deficiency are recorded in a logbook at each site and when possible appropriate action is taken to correct the abnormality.

Disease severity is estimated by comparison with published standards (C. James, A manual of assessment keys for plant diseases, Canada Department of Agriculture, Publication No. 1458. 1971) and/or estimated as a percentage of total leaf area affected (see Table 1 for other QA information).

4. Area Measurements

Leaf area measurement is taken with a meter that photo-electronically detects and measures the amount a sample shades a scanning light beam. See Table 1 for standard operating procedures.

Calibration checks are conducted at the beginning and end of each measuring session. During lengthy (4 hours or more) sessions, a mid-point calibration check is made. Test plates of known area are available for operator calibration checks For each check, one calibration test plate is measured. If the reading of the test plate exceeds ± 5% of the known area, belt cleanliness, tracking, and tension are checked. If the observed area of the test plate still exceeds ± 5% of the known area, the site BQAL is contacted to initiate corrective action. This can include adjusting the meter, calibration settings, or calling a qualified equipment representative.

Once proper calibration is achieved, the observed mean area and mean % difference of the test plate is recorded on the verification form entitled "Quality Assurance Calibration Checks for Area Measurements" (Figure 4).

Precision is determined by duplicating the measurement of every 30th sample, or a minimum of 3, whichever is larger. A notebook is kept of all calibrations, precision tests, and adjustments.

QUALITY ASSURANCE CALIBRATION CHECKS FOR AREA MEASUREMENTS

Area Meter _____

$$\text{Mean \% Difference} = \frac{\text{Mean Observed Area - Known Area}}{\text{Known Area}} \times 100$$

Date	Project	Operator Initials	Start-up comments regarding condition of transparent belt			Start-up Check			End of session Check			End of session comments regarding condition of area meter. Operator please initial.
			Cleanliness	Tracking	Tension	Mean (cm^2)	Known (cm^2)	Mean Diff	Mean (cm^2)	Known (cm^2)	Mean Diff.	

Figure 4

5. Physiological Measurements

Plant physiological measurements include:

a. leaf water potential

b. leaf conductance

c. leaf transpiration

d. photosynthesis

e. chlorophyll

These measurements are instantaneous readings of plant processes which are not amenable to precision and accuracy statements and for which no standards have been developed. It is not possible to determine what level of process a particular plant should display at any point in time. In making duplicate measurements of physiological conditions, it is not possible to determine if the measurements are being taken at the exact same point or if the same level is being measured. Plant process levels vary from point to point within a plant and can change rapidly over time.

Researchers at each NCLAN location follow and document (in instrument logbooks) manufacturers' standardizations, checks, and procedures (Table 1) to ensure proper operation and maintenance of instruments used in making physiological measurements. All of the above measurements may not be necessary in every study. Due to funding restrictions, it may not always be possible to use the same manufacturer and model of instrument for a particular process at all sites.

5.0 QA Data Handling Procedures

5.1 Field -- Collection Handling Procedures

For verification and tracking purposes, data is identified by:

1. location
2. year
3. crop(s)
4. pollutant(s)

Biological data collection for weights, soil moisture, photosynthesis (UCR only), leaf area, disease severity, leaf water potential, and chlorophyll content are manually recorded on data sheets. Data flow and verification checks for these measurements and observations are shown in Figures 5, 6, and 7. Data sheets and/or data notebook checks are made by the principal investigator or BQAL at each site to ensure that manually collected data are entered correctly. The last page of data on each harvest or group is initialed by the principal investigator or the BQAL.

Data collected automatically for leaf conductance, leaf transpiration, and photosynthesis are read directly into a microprocessor in the field. Data flow and verification checks for these measurements are shown in Figure 8. Computer files for all biological data (manually and automatically collected) are checked by the principal investigator or the BQAL to ensure that the data fields are complete and the recorded values are reasonable before data analysis is started.

All data sheets, punch cards, and data tapes are maintained for at least 3 years by the BQAL at each location.

Plant weights, leaf area, height, plant water potential, chlorophyll, injury, soil moisture, etc.

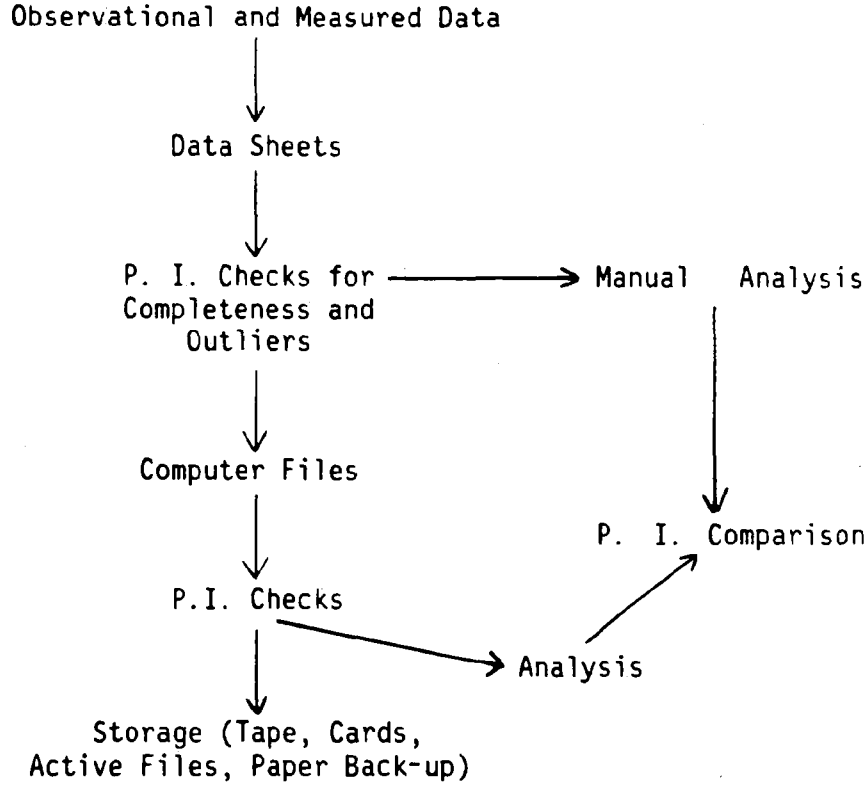

Figure 5. Data Flow and Verification for Manually Collected Biological Data at ANL

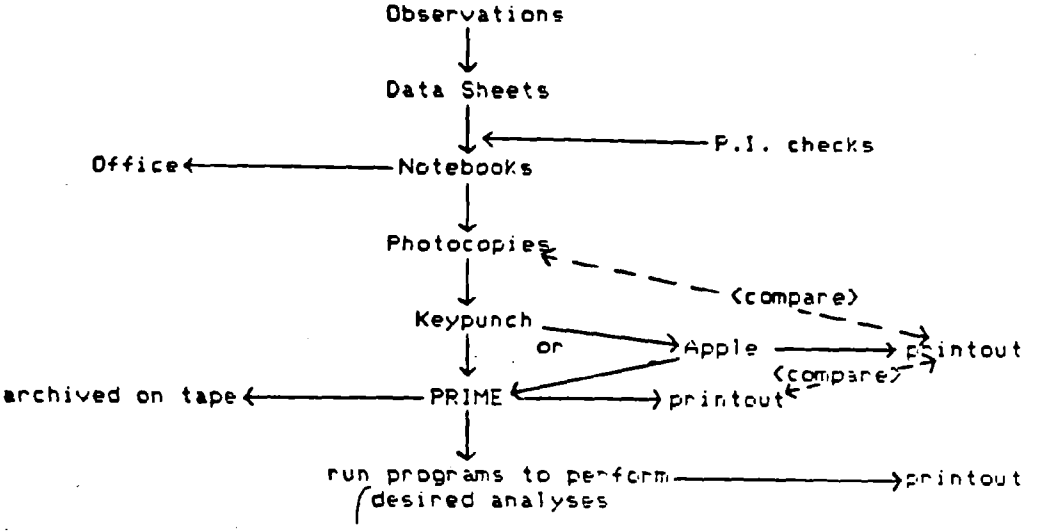

Figure 6. Data Flow and Verification for Manually Collected Biological Data at UCR

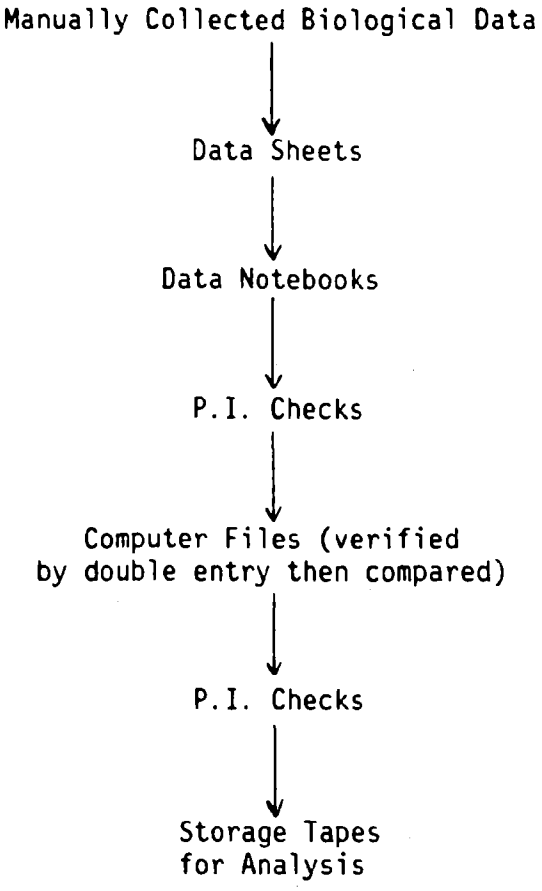

Figure 7. Data Flow and Verification for Manually Collected Biological Data at BTI and RAL

Photosynthesis and Stomatal Conductance

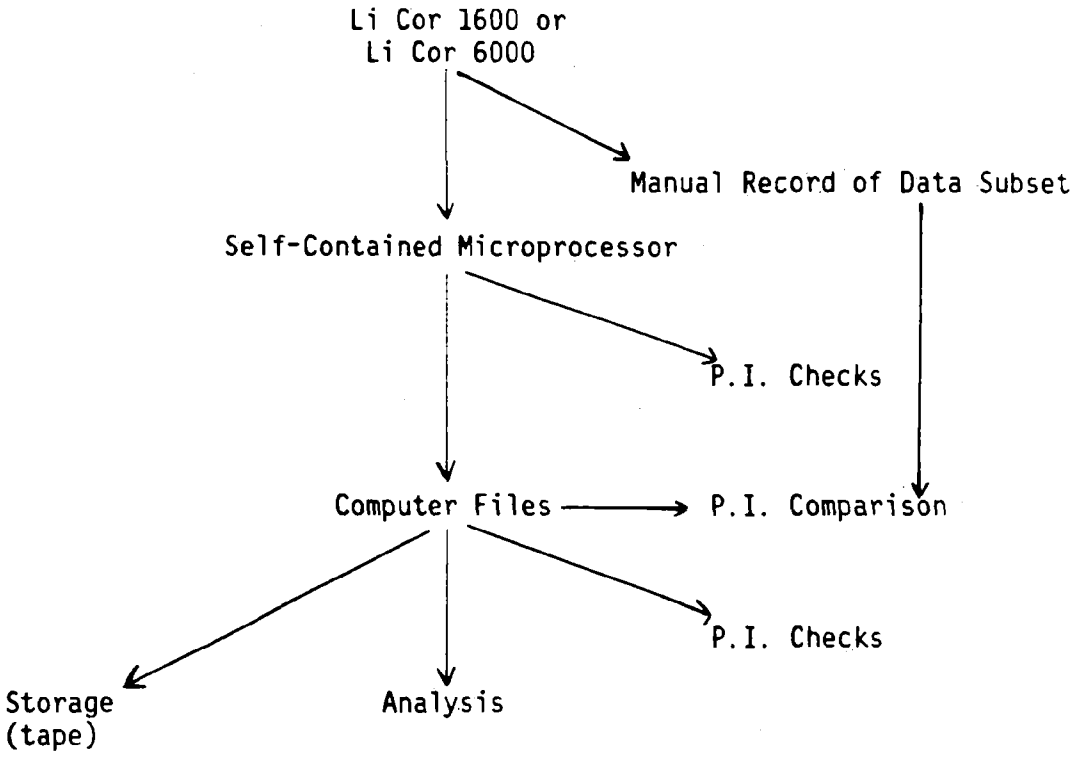

Figure 8. Data Flow and Verification for Automatically Collected Biological Data for All Locations

Data analysis is conducted at each site using the Statistical Analysis System (SAS) software package (ANOVA, regression, etc.) and the Weibull function model as described by Heck et al., Environ. Sci. Technol. Vol. 17, No. 12, pp. 572A-581A, 1983; and Rawlings and Cure, Crop Science, In press, 1985.

5.2 Data Synthesis and Analysis

For verification and tracking purposes, all sets of NCLAN biological data collected by the NCLAN data library at NCSU in Raleigh are identified using the following descriptive information.

General: Site, Year, Crop (common, Latin, cultivar)

Cultural: Dates (planting, emergence, start and end of exposure with growth stage), soil type and name, row and plant spacing)

Exposure and Monitor Information: Pollutant and method of addition, start and stop times each day, number of exposure days, description of sequential monitoring.

APPENDIX

Table 1. Summary of NCLAN Biological Quality Assurance

Parameter/Method	Sites	Standard Operating Procedure Citation	% Precision Required	% Accuracy Required
1. Plant Weight				
a. Balance	ALL	See weight measurements section of this document	± 8	± 1.5
2. Soil Moisture				
a. Depth Neutron Probe	UCR, RAL, ANL	1. UCR -- Troxler 3220 Instruction Manual: 1981 Section 2-1 to 2-6 and 4-1 to 4-2.	N/A	N/A
		2. RAL -- 503 DR Hydroprobe Moisture Depth Gauge Manual: May 9, 1984 Operation: Section 2, pp. 6-20 Calibration: Section 3, pp. 30-32	N/A	N/A
		3. ANL -- Troxler 3222 Instruction Manual: 19__ Section 2-4 to 2-6	N/A	N/A
b. Surface Neutron Probe	ANL	Troxler 3411-B Series Surface Moisture-Density Guage Manual Operation, pp. 8-9	N/A	N/A
c. Tensiometer	RAL, BTI, ANL	Irrometer Co. Reference Book #24, pp. 2-14. Also see Soil Moisture Measurements this document	N/A	N/A
3. Plant Health				
a. Visual Examination	ALL	Disease -- C. James, A manual of assessment key for plant diseases, Canada Dept. Agric. Pub. No. 1458, 1971	N/A	N/A
4. Leaf Area				
a. Area Meter	ANL	Portable Licor LI-3000	± 10	± 5
	BTI	Licor LI-3100 and 3000	+ 10	± 5
	RAL	Licor LI-3100	+ 6	± 3
5. Leaf Water Potential				
a. Pressure Bomb	UCR, ANL	Plant Water Status Console 3000, Instruction Manual 1981, pp. 5-9	N/A	N/A
	BTI	PMS Pressure Bomb	N/A	N/A
b. Thermocouple Psych	RAL		N/A	N/A
6. Leaf Conductance				
a. Steady-State Porometry	UCR& BTI	Licor LI-1600, Instruction Manual 1982, pp. 4-1 to 4-12 and 5-1 to 5-10		N/A

(continued)

Table 1 (continued)

Parameter/Method	Sites	Standard Operating Procedure Citation	% Precision Required	% Accuracy Required
b. Rate of Water Accumulation	ANL	Li-Cor LI-6000	N/A	N/A
7. Leaf Transpiration				
a. Steady-State Porometry	UCR& BTI	Li-Cor LI-1600, Instruction Manual 1982, pp. 4-1 to 4-12 and 5-1 to 5-10	N/A	N/A
b. Vapor Pressure Difference	ANL	LI-COR LI-6000, Instruction Manual 19__, pp. 2-1 to 2-8		
8. Photosynthesis				
a. Isotope Porometry	UCR	UCR mfg. Johnson et al. (1979), Photosynthetica 13:403	N/A	N/A
b. CO_2 Depletion	ANL	Li-Cor LI 6000	N/A	N/A
9. Chlorophyll a.	ANL BTI	-- Arnon, D. J., 1949, Plant Physiology 24:1-15	N/A	N/A