PART C

WATER STATUS AND CARBON DIOXIDE EXCHANGE STUDIES

PRESSURE CHAMBER TECHNIQUES FOR MONITORING AND EVALUATING SEEDLING WATER STATUS

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ABSTRACT

Pros and cons of using the pressure chamber technique for evaluating seedling water status are presented. Safe use of the instrument, sample preparation, and proper operation to obtain the best possible data are emphasised. Guidelines for determining and interpreting water potential (ψ_w) levels are given for bare-root and planted seedlings. Results from two Oregon studies concerning the effect of water potential on seedlings show the pressure chamber to be extremely useful in monitoring ψ_w at different stages of reforestation.

INTRODUCTION

Use of the pressure chamber to evaluate plant water status has increased dramatically during the past decade. First introduced in 1914 (Dixon, 1914), the technique was virtually ignored until 1965 when Scholander reintroduced it (Scholander *et al.,* 1965). Since then, plant water status generally has been determined by the pressure chamber—or "pressure bomb"—technique. Beginning in 1968, foresters in the Pacific Northwest have used this method extensively to evaluate seedling water status (Cleary, 1968).

Comparisons between the pressure chamber method and other techniques of measuring plant water potential are numerous (Boyer, 1967; Kaufman, 1968; Ritchie and Hinckley, 1975) and have validated using the pressure chamber as both a research tool and practical device. Fast, reliable, and accurate measurements can be made easily in the laboratory or field. However, it is not our intent in this paper to review all the techniques for estimating plant water status (see Ritchie and Hinckley, 1975, for a summary of comparisons published up to 1974) but, instead, to discuss the merits and safe, effective use of the pressure chamber method.

The J-14 press has been suggested as a substitute for the pressure chamber.[1]

[1] S. Childs. 1979. Evaluation of a rapid technique to estimate plant water potential for applications in forests. (unpubl.).

Basically, this device uses a flexible membrane to apply pressure to a tissue sample. Observed through a plexiglass window, the sample will either exude water from its xylem elements or, at lower water potentials, change colour as cells rupture. The pressure required to cause either of these changes is calibrated to some other measure of water potential, often a pressure chamber. Our attempt to calibrate the J-14 using the pressure chamber as suggested by Childs[1] gave an unusable calibration curve (Fig. 1). Even though the J-14 can make measurements rapidly and does not require a compressed gas supply, it has some serious disadvantages: (1) the endpoint is indefinite; (2) precision is low; (3) it requires calibration with a pressure chamber; and (4) different age foliage gives different results. A very large sample size would be required to overcome these shortcomings and achieve an acceptable degree of accuracy.

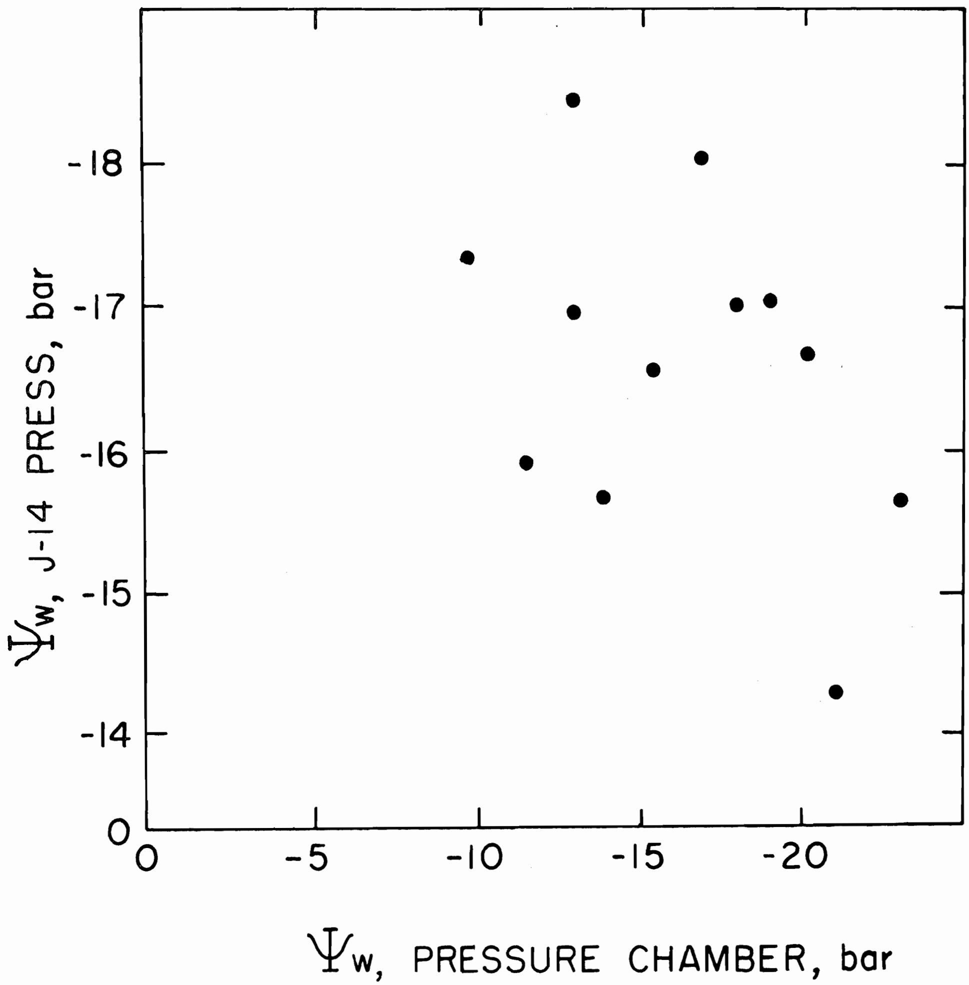

FIG. 1—Calibration data for the J-14 press for 12 paired samples measured simultaneously with the J-14 press and pressure chamber techniques.

USING THE PRESSURE CHAMBER

Foliage cut from a branch (the sample, A) is placed in the chamber (B) with the cut end of the sample exposed through the chamber cover (Fig. 2). Chamber pressure is slowly increased with nitrogen from a high pressure tank (C) until water is forced back to the cut surface (D). That pressure, indicated on the pressure gauge (E), estimates water potential (ψ_W). Scholander *et al.* (1965) proposed that this technique measures gravitational and functional potentials. Boyer (1969) demonstrated that xylem water potential is indeed equal to the combined gravitational and functional components plus a third component comprising xylem solute and matric potential. This third component is not measured with the pressure chamber technique. Because it is commonly either negligible or constant, this correction for matric potential is generally ignored and the pressure chamber measurement accepted as an estimate of ψ_W.

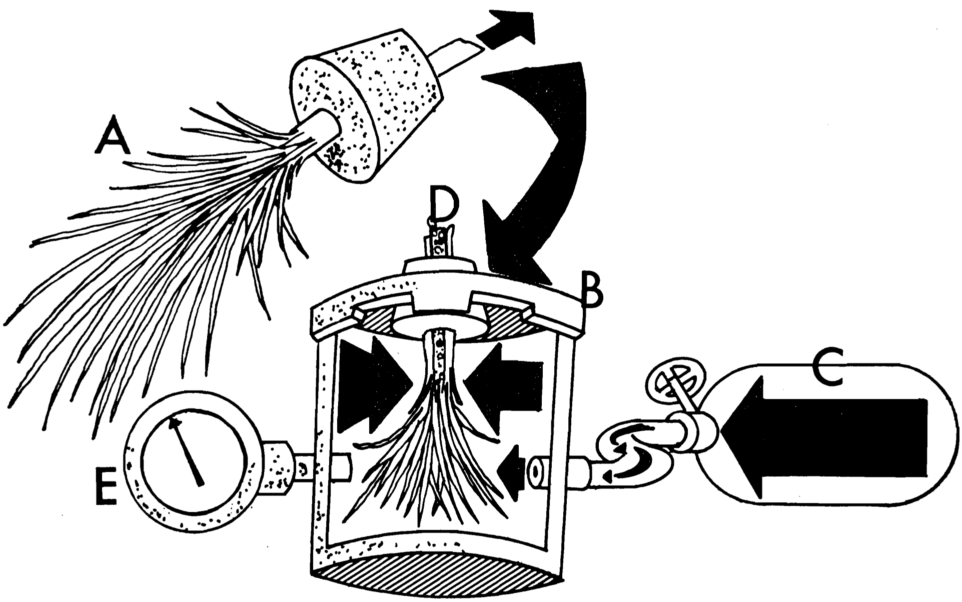

FIG. 2—Measuring ψ_W with a pressure chamber. A, sample; B, pressure chamber; C, pressure tank; D, water forced back to cut surface; and E, pressure gauge.

Safety Considerations

The pressure chamber apparatus is a potentially dangerous device which should be treated with great respect. It can be extremely useful but also can injure a careless operator. Because the device uses compressed inert gas (preferably nitrogen) as its pressure source, failure of the pressurised vessel may cause the sudden release of pressure similar to an explosion. Sudden failure of the connecting hose or tubing, though less serious, could result in a wildly thrashing hose or, possibly, flying parts.

To operate the pressure chamber safely, establish and use the following routine for applying pressure to the instrument:

(1) Turn the instrument's control valve to "off".

(2) Check the supply hose to see that it is connected properly. Check, by careful inspection and tugging, hose fittings and any other connections that might be loose.

(3) Open the valve on the supply tank *slowly,* not suddenly. If the system should fail anywhere, the more nearly closed this valve is, the less damage is likely to result. This same principle — open the valve slowly — also applies to filling a portable tank from a main supply tank.

(4) Do not turn the valve wide open! Instead, open it only wide enough to permit the chamber to be pressurised without a substantial drop in hose pressure. One-half turn is usually sufficient. Close the main tank valve at the end of the day to conserve gas in case a small leak develops.

(5) Test the instrument's safety equipment to help detect leaks and perform minor adjustments on valves. Clean and lubricate chamber seals because dirt and foliage cause wear and can even plug valves or tubing.

Following these safety guidelines will not only minimise the inherent hazards of the technique but will also improve the efficiency of collecting data.

Sample Preparation

Preparing a sample for the pressure chamber will most often involve removing a small lateral branch; very small seedlings may require using the entire top. Removing the sample with a sharp knife or razor blade eliminates needless recutting, which can introduce error in species with large xylem vessels (Scholander *et al.,* 1965).

In woody species, the bark must be stripped back far enough to allow the woody portion to protrude through the rubber sealing gasket, where the cut surface can be observed. Failure to remove the bark may result in mistaking phloem exudate for xylem sap at the endpoint. Resin can also obscure the endpoint in species with resin tubes, particularly the pines. Resin usually forms bubbles which break and can be wiped away to allow observation of the xylem endpoint.

Approximately 90% of the sample should be inserted inside the pressure chamber to avoid introducing measurement error (Waring and Cleary, 1967). Additional error may result if large proportions of a small, succulent sample are squeezed by the gasket, a situation that must be carefully evaluated when the sample is a conifer needle. For example, ψ_w can be successfully measured with a fascicle of pine needles, but substantial (5- to 8-bar) errors in measuring Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) needles can occur even with a gasket only 4 mm thick (Cleary, 1971). Samples from dormant seedlings can be stored for 20 minutes under humid, low-stress conditions with little error introduced; however, seedlings actively growing in hot, dry conditions can easily read 10 bar lower after the same 20-minute period. Therefore, standardising measurement techniques based on sound physical and physiological principles is essential. Experience in using the instrument is also helpful in obtaining dependable and accurate data.

Operation

1. Never place any part of the body — particularly the eyes — directly above the hole in the chamber cover. Because fairly high pressures are required to force a plant through the seal, such an event can occur unexpectedly and with considerable vigour. The endpoint can be observed just as easily from the side as from above.
2. Never leave the chamber cover or lid half on. It should always be either on and ready to be pressurised or completely off. A cover sitting on the chamber but not locked in place can form enough of a seal to withstand low pressure, but may blow off at higher pressures. Avoid this situation by always leaving the chamber cover all the way on or all the way off.
3. After completing the day's measurements, close the main tank valve and leave the control valve on off or exhaust. Again, leave the cover all the way on or all the way off. If removing the supply hose is necessary, ascertain that the hose is not pressurised *before* disconnecting it. Like the chamber cover, quick-disconnect fittings should be either completely connected or completely disconnected. Connections should be made only when the hose is *not* pressurised.

EVALUATING WATER POTENTIAL IN SEEDLINGS

Field experience and research studies on the growth and survival responses of seedlings to different water potentials has led to establishing guidelines for conifer seedlings. These guidelines, based primarily on data collected for Douglas fir and ponderosa pine (*Pinus ponderosa* Laws), generally have been applied to all Pacific Northwest conifers (Cleary and Greaves, 1978). We plan to conduct experiments that may refine these guidelines for other species, particularly western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western redcedar (*Thuja plicata* Donn), and true firs (*Abies* spp.).

Bare-Root Seedlings

Seedlings removed from a nursery bed should be watered immediately to reduce water stress. Maintain a ψ_w level above — 10 bar at all times thereafter. Ideally, bare-root seedlings should have a ψ_w of greater than — 5 bar; if ψ_w decreases to — 10 bar, the risk of planting a damaged tree is significant; if ψ_w decreases to — 20 bar, the likelihood of severe physiological damage to root tissue is so high that seedlings are probably best destroyed. A ψ_w less than — 50 bar is probably lethal for most seedlings. Because water potential can be raised immediately by adding water to seedlings in a packing bag, ψ_w should be evaluated before water is added. The maximum observable value with the pressure chamber is — 1 to — 2 bar in Douglas fir and slightly higher in ponderosa pine.

Planted Seedlings

For seedlings planted in soil or other media, ψ_w is not limiting for photosynthesis at levels above — 10 bar (Cleary, 1971). In ponderosa pine, at ψ_w levels between — 10 and approximately — 20 bar, seedling photosynthesis is slowed or stopped; between — 20 and — 50 bar, seedling vigour continually declines; and at levels below approximately — 50 bar, seedlings die (Fig. 3). The lower the ψ_w, the longer it takes for seedlings to recover after watering (Fig. 4). In Douglas fir, lack of water does not limit photosynthesis above —10 bar; between —10 and approximately —30 bar, photosynthetic capacity of seedlings declines; and between —30 and —50 bar, seedling vigour drops. Death occurs at ψ_W levels in excess of —50. Other species have similar responses but at different values of ψ_W. An estimate of seedling vigour in response to ψ_W is essential to interpret the effect of low water potential on seedlings.

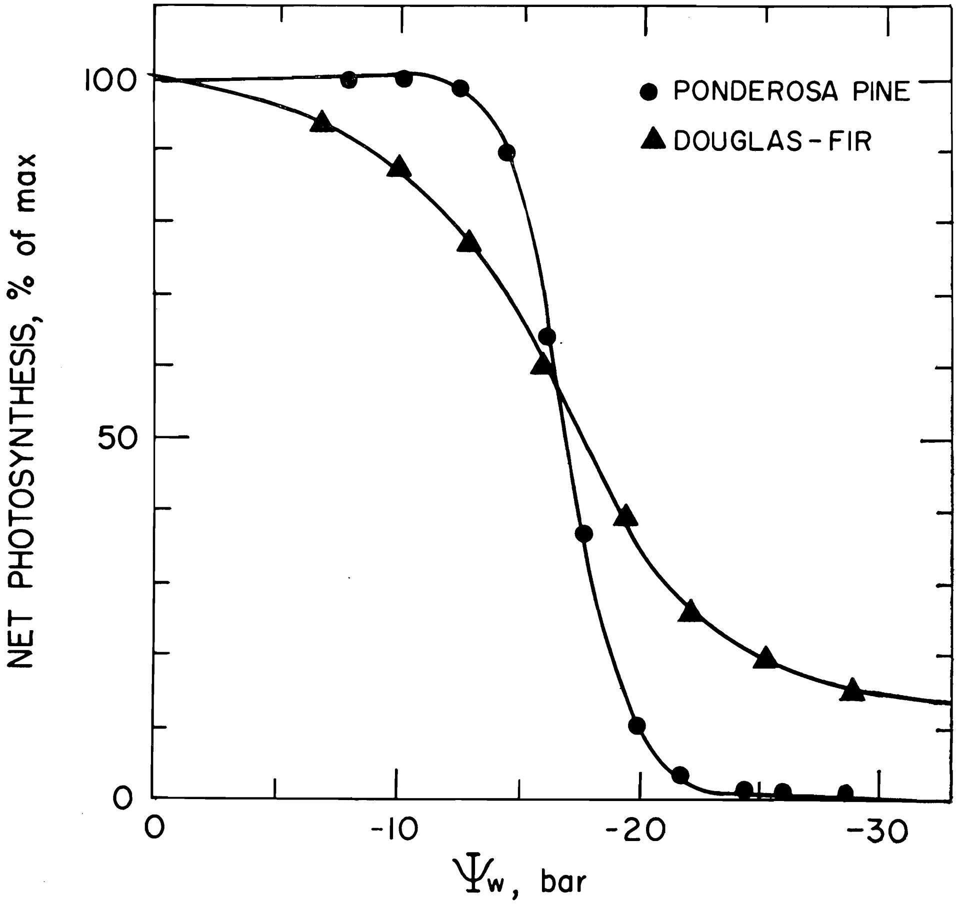

FIG. 3—Photosynthetic response of Douglas fir and ponderosa pine seedlings to decreasing ψ_W (Cleary, 1971).

Monitoring Bare-Root and Planted Seedlings: Two Example Studies

Two recent Oregon studies have demonstrated the usefulness of monitoring water potential at different stages of reforestation.

The first study examined the effects of winter ψ_W on bare-root Douglas fir seedlings during nursery lifting and processing (Daniels, 1978). Daniels evaluated three groups of seedlings each lifted at a ψ_W of either —4, —12, or —20 bar during mid-February. Half the seedlings undercut at —12 and —20 bar were not watered after lifting (dry

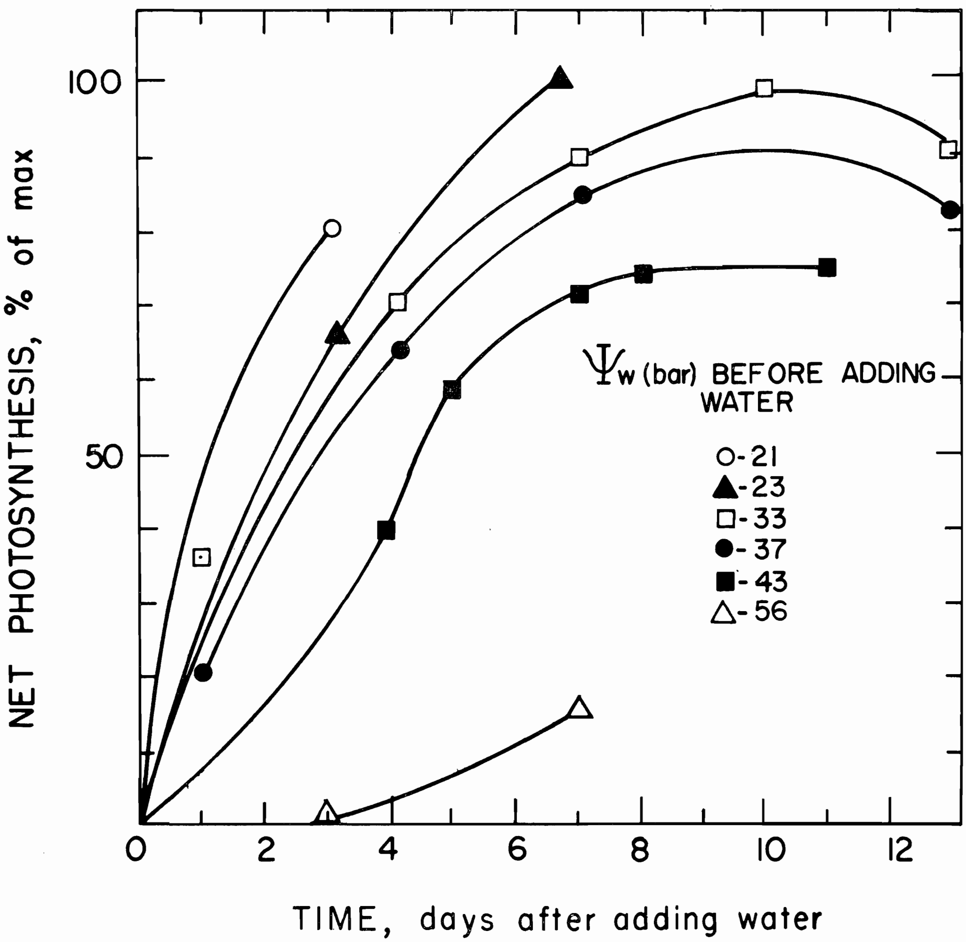

FIG. 4—Photosynthesis of ponderosa pine seedlings as a function of time after watering (Cleary, 1971).

treatment). Water is seldom added at lifting in nurseries where dry conditions are not prevalent throughout most of the lifting season. The other half had shoots and roots directly sprayed with water immediately after lifting (wet treatment). It is significant that the dry-treated seedlings lifted at —12 bar had decreased to less than —20 bar ψ_w within 2 hours after lifting. Thus, essentially no difference existed between the —12- and —20-bar dry-treated seedlings because of the nursery processing technique. Wet-treated seedlings grew significantly taller (9 cm compared with 8 cm) and produced more new foliage (5.7 g compared with 4.77 g) than dry-treated seedlings when grown free of competing vegetation in a nonstressed environment. Survival without competition was excellent for all treatments, ranging from 93 to 98%. In contrast, when outplanted on a stressed site with considerable competition between grass and seedlings, 43% of wet-treated seedlings survived compared with 23% of dry-treated seedlings. Wet-treated seedlings also grew taller (7.6 cm compared with 6.6 cm) and produced more new foliage (3.1 g compared with 2.6 g). Therefore, stock protected from desiccation during lifting and processing in accordance with the previously outlined guideline survived better and grew faster during the year after planting (Cleary and Greaves, 1978).

The second study involved irrigation scheduling at a major Oregon nursery. Lavender and Cleary (1974) outlined conifer seedling-production techniques to improve seedling establishment and identified the need to ensure that dormancy induction be initiated early (by mid-July) in the summer. Seedlings must encounter moderately low water potentials to induce dormancy (Lavender and Cleary, 1974). The key to doing so is having plants large enough early in the summer so that nurserymen are not forced to continue irrigation merely to attain some size objective. Oregon nurseries should seed early in the spring at a bed density low enough to ensure high growth rates early in the growing season. For the first year, irrigation must be frequent during the early growing season to maximise growth until approximately mid-July. After 1 August, irrigation should be used only on extremely hot days (35°C) or when early morning ψ_w is below — 12 bar. After 1 September, ψ_w should be allowed to reach, but not exceed — 15 bar until autumn rains begin. From 1 August to the autumn rains, irrigation should be minimal or sufficient only to bring temporary relief of successively lower ψ_w levels, and should not saturate the soil profile. During the second growing season or the growing season of a transplant crop, schedules should be such that irrigation during the early part of the season is frequent enough to keep the soil profile near field capacity and yet not saturated until mid-June. Thereafter, early morning ψ_w levels should be allowed to decrease to — 15 bar; again, irrigations should be short so that ψ_w will remain above — 10 bar but frequent enough to prevent it from exceeding — 25 bar. The ψ_w guidelines outlined previously are useful in implementing the techniques for improving seedling establishment.

SUMMARY

Seedling water status is a critical physiological function that can easily be measured with the pressure chamber. As with any device using highly pressurised gas, safe and careful operation is a must. High ψ_w levels — determined by the pressure chamber — ensure vigorous seedlings, and thus high survival potential. The measurement of ψ_w is a point sample, however, and cannot detect previous desiccation damage in a plant rewatered after damage has occurred. Maintaining high ψ_w levels between lifting and outplanting is an important part of producing high quality stock and is essential to maintaining high survival and growth rates of outplanted seedlings.

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