Monitoring Cold Hardiness of Tree Seedlings by Infrared Thermography[1]

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Abstract.--In this first known attempt, infrared imaging was used to relate foliage temperature to dormancy and degree of cold hardiness in tree seedlings. Species studied were Engelmann spruce, ponderosa pine, and Douglas-fir from northern Arizona. Preliminary results suggest that the dynamic responses of the foliage to changes in light (on/off) are potentially related to degree of cold hardiness. Until these initial results are confirmed in more exhaustive studies, and understood, infrared thermography cannot be recommended as an operational tool for seedling evaluation.

INTRODUCTION

For successful plantation establishment, tree seedlings must be in proper condition to survive the shock of outplanting and to establish a root system in intimate contact with the soil. The condition of a seedling at any time is determined by the interaction of its genetic capacity and the sequence of events to which it is exposed, including the magnitude, timing, and duration of the environmental conditions (Jenkinson and Nelson 1984).

Even if it were possible to know in detail the entire history of the seedling, current understanding of plant physiology would not allow a precise determination of seedling capacities from that history. For this reason there has been a significant amount of effort spent over the last few decades looking for a test or measure of seedling condition that is related to, or predictive of, a response of interest. For the nursery manager, the response of interest could be the capacity to grow new roots at a rate sufficient to ensure seedling survival and rapid early growth following outplanting. The time of interest could be when the seedlings in the nursery enter the physiological condition that makes them capable of being lifted, processed, and outplanted and still retain the capacity to respond favorably in the field. For the regeneration forester, the response of interest may be the same, but the time of interest is later, after everything but planting has become part of the seedling's history. In both cases, once seedling condition is known, decisions can be made and options chosen based on a reasonable anticipation of outcome. To be useful, however, such knowledge must be readily available in time to make the decision or choose the option.

The test applied must meet several criteria. It must be reliable, reasonably easy to use, and not particularly subject to error. In addition, it must be based on principles, processes, or relationships that are sufficiently "known and understood" so that variations in test conditions and results can be interpreted.

This paper reports a study of infrared thermography as a potential means of identifying when tree seedlings change physiological state (e.g., change in dormancy status or frost hardiness level).

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INFRARED THERMOGRAPHY

Using thermal imaging technology currently available commercially, it is possible to create a clear and precise TV-quality image based on surface temperature patterns within a scene. Temperature differences of as little as 0.1°C are apparent, and quantitative data can be extracted from the image.

The major plant factor affecting seedling temperature under a given set of environmental conditions is stomatal conductance (a function of stomatal aperture), because of its effect on transpiration rate and therefore the degree of evaporative cooling. Stomatal conductance is affected by several plant and environmental factors--e.g., plant water stress, light intensity, relative humidity--that usually vary over relatively short time periods. In addition, however, some evidence suggests that stomatal behavior varies over longer, phenology-related periods, perhaps associated with state of dormancy and cold hardiness. Most of this evidence suggests that stomata are more nearly closed (decreased conductance) and respond less to environmental factors including light, when the seedling is dormant (Christersson 1972; Kozlowski 1943; Parker 1963). However, some trees (e.g., coastal Douglas-fir) increase stomatal conductance (stomata more open) during the winter, and their stomata remain open at night (Murphy 1979; Running 1976). If it were possible to isolate seedling temperature patterns attributable to phenology-related changes in stomatal behavior, infrared thermography might be developed into a useful tool for evaluating seedling dormancy or cold hardiness or both.

Current infrared technology is sensitive, easy to use, does not affect the seedling in any way, and produces images that potentially contain far more information than would a single point measurement of temperature or transpiration rate. Preliminary observations of several coniferous species suggested that seedling temperature (as measured by thermography) varies in relation to length of tine since beginning of emergence from the last stage of dormancy (Weatherspoon and Laacke 1985).

Thermography could be used for other purposes such as monitoring effectiveness of irrigation systems through effect on relative water stress (Vicek and King 1983).

Stage 1	Stage 2	Stage 3	Stage 4
3 weeks	7 weeks	5 weeks	4 weeks
10-hr day 20°C day 15°C night	10-hr day 10°C day 3°C night	10-hr day 5°C day -3°C night	16-hr day 22°C day 22°C night

Figure 1.--Environmental conditions during the four stages of the 19-week regime. Stages 1 through 3 were successive steps in - hardening, stage 4 was the dehardening time.

METHODS

This study was designed and implemented as an addition to the ongoing work of Dr. Richard Tinus of the Rocky Mountain Forest and Range Experiment Station, USDA Forest Service.

Seedlings of Douglas-fir (Pseudotsuga menziesii var. glauca LBeissn.] Franco), Engelmann spruce (Picea enaelmannij Parry ex Engelm.), and ponderosa pine (Pinus ponderosa var. seopulorum Engelm,) were greenhouse grown in 400 ml Rootrainers, one seedling in each of the four compartments of the folded container, or "book." Beginning in June, 1985 they were moved to growth chambers where photoperiod and day/night temperatures were altered in four stages over a period of 19 weeks, first to induce cold hardiness and then to promote dehardening (fig. 1). A full description of the environmental conditions maintained during each stage, as well as the physiological results of the hardening and dehardening regime, are described in papers by Tinus et al. and Burr et al. elsewhere in these proceedings.

Apparent foliage temperatures were measured with an Inframetrics 525 Imaging Radiometer at the time of each scheduled stage change. At each measurement time, infrared images of seedlings were recorded on video tape which, along with appropriate instrument data and verbal notes, permitted later quantification of seedling temperatures.

Seedling temperature is affected not only by stomatal conductance, but also by a number of environmental variables. To try to minimize the effects of these other variables, we made most of the thermographic measurements in a separate "measurement" growth chamber maintained as nearly as possible under constant environmental conditions. Temperature was set at 20°C

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[4] Tradenames and commercial products and enterprises are named solely for information. No endorsement by the U.S. Department of Agriculture is implied.

during both light and dark periods. Relative humidity generally ranged from 25 to 35 percent. During light periods, sodium and mere ry vapor lamps provided about 150 uEs^-1 m $^{-2}$ of PAR (photosynthetically active radiation) at seedling height. This level of radiation permitted near-maximum stomatal conductance in actively-growing, well-watered seedlings, yet minimized temperature differences between shaded and directly irradiated portions of a seedling.

Eight seedlings of each species were removed from the conditioning environment at the end of each stage throughout the hardening/dehardening cycle and placed in the measurement chamber. Infrared temperature measurements were begun the day following removal and were continued for 2 days. During this time thermographic measurements were taken through transition periods from lights off to lights on, and from lights on to lights off. Measurement began before light conditions were changed and continued for up to 3 hours. The seedlings were then placed in the root growth capacity test described by Tinus et al. (these proceedings).

For measurements in the chamber, the sensor was mounted on a remotely controlled swivel and the seedlings were arrayed in a semi-circle equidistant from the swivel point. Air temperature in the chamber was monitored in three ways: (1) A dial thermometer was inserted through the wall of the chamber and into the air flow before it passed over the seedlings. Temperature indicated on this thermometer was recorded at the beginning of each sensor scan. (2) A recording hygro-thermograph was placed in the chamber in the center of the semi-circle of trees and below the level of the foliage. Air temperature and relative humidity were recorded continuously on a 7-day chart. (3) Additional temperature references, readable by the infrared sensor, were placed in the chamber to provide a real-time indication of air temperature in the vicinity of the seedling and as a visual reference in reviewing the video tapes. These non-standard temperature references, or "wicks," were wedges of filter paper held by one end in plastic soda straws and displayed as fan-shaped objects close to the foliage of the trees to be measured. One was kept wet by immersing one end in a bottle of distilled water. This was intended to provide, along with the temperature of a similar but dry wick, a reference to relative humidity. Ultimately, only the temperature of the dry, white wicks was used as the thermal reference for air temperature. In this report, all foliage temperatures are described as degrees centigrade above or below air temperature.

During the dehardening stage, supplementary measurements were taken on a table outside the measurement chamber. For the first 2 weeks of the dehardening stage, these measurements were taken daily to monitor anticipated rapid changes in physiological condition of seedlings. For the time required to take the measurements, seedling containers were placed on a rack inside a large 3-sided cloth enclosure open at top and bottom. This was done to measure seedling temperature responses without the forced air flow--unavoidable within the chamber--that tended to mask the effects of stomatal conductance on seedling temperature. A bank of lights--producing 4000 lux of cool, white, fluorescent light--was suspended 60 to 90 cm (2 to 3 ft) above the seedlings. A shielded mercury thermometer was suspended just above the seedling tops. As in the measurement chamber, dark-to-light and light-to-dark transitions were monitored.

When the dehardening stage began, a supply of seedlings of each species was held in conditions of the third stage of hardening (fig. 1). Two books of four seedlings of each species were removed at about 2-day intervals and placed in the dehardening conditions of stage 4 (fig. 1). This provided the opportunity to test seedlings at three different states of dehardening at the same moment and under the same conditions. Measurements were continued daily for 10 days.

RESULTS AND DISCUSSION

Hardening Period

At the end of the greenhouse phase, and before seedlings were placed in stage 1 hardening conditions, foliage/air temperature differences were near zero during dark periods for all species. When lights were turned on, foliage temperatures dropped to between 1° and 2°C below air temperature. In successive light periods no one species was consistently warmer or cooler than the others. The general pattern of temperatures is consistent with the type of stomatal behavior expected in actively growing plants.

At no other time in the 19-week regime did foliage temperatures warm up to air temperature in the dark, even after 16 hours of uninterrupted darkness during dehardening. The time when foliage temperatures returned to air temperature corresponded with the only time in the 19 weeks that fully matured, current-year's foliage, as yet unexposed to hardening conditions, was present.

At the end of the first stage of hardening, differences between species in general foliage temperature appeared. Ponderosa pine and Douglas-fir were similar and both warmer, on the average, than Engelmann spruce. During light-to-dark and dark-to-light transition, ponderosa pine and Douglas-fir foliage temperatures overlapped.

At the end of the second stage of cold hardening, relative temperature of Engelmann spruce fell farther below the other two species. On the first day after removal from the treatment chamber, ponderosa pine was warmer than Douglas-fir. By the second day, ponderosa pine was cooler than Douglas-fir, and Engelmann spruce became even cooler relative to the other two. A possible explanation for the change in relative position of the species is that low soil temperature in the container (residual from the induction treatment) affected ponderosa pine more than Douglas-fir, and the resulting increase in resistance to water uptake and movement reduced the amount available for transpiration and, therefore, increased the tissue temperature. By the second day, sufficient warming could have occurred to allow pine to absorb and transport water more easily. This relative difference would be consistent with data on other species that indicate a greater effect of cold soil on low elevation or low latitude sources relative to those from higher elevation or higher latitude (Kozlowski 1943; Kramer and Kozlowski 1979) and with the relative elevations of the sources of all three species.

At the end of the third stage of cold hardening, relative temperatures of the species changed again. At this time Douglas-fir and Engelmann spruce had equivalent temperatures and both were warmer than ponderosa pine.

On the first day after placement in dehardening conditions, the foliage temperatures of all three species were equivalent. However, after 4 weeks in uniformly warm temperatures, Engelmann spruce had again assumed, on the average, a lower temperature than the other species.

It was obvious during the three cold hardening stages that foliage temperature varied from place to place on seedlings of all species. A common situation, especially for Engelmann spruce, was an area of foliage that was distinctly cooler than the rest of the seedling. Occasionally areas would develop that were distinctly warmer than the rest of the seedling. For example, branch tips of Engelmann spruce were occasionally up to a degree warmer than the general foliage. Temperature patterns periodically developed on seedlings with a range of 1.3° to 4.3°C difference between the warm areas and the cool areas.

Dehardening Period

More detailed data were gathered during the dehardening period because it was possible to measure the same seedlings repeatedly over an extended time. During the transition time from maximum to minimum cold hardiness (time of bud break), response of seedlings to light changes varied at different stages of dehardening. By the time the process was complete, seedlings within each species responded very much alike (fig. 2).

If physiologically reactive stomates tend to close in the absence of light, transpiration should be reduced and foliage temperature should increase relative to air temperature when lights are turned off. Until Douglas-fir seedlings had lost an estimated 30% of their acquired cold hardiness, the temperature of the cool areas on the seedlings decreased or remained unchanged relative to air temperature when the lights were turned off. After losing about 30% of the total hardiness gained, temperature of the cool portions of the plant increased in the absence of light.

General temperature response patterns to changes in light (on or off) did not change although differences between foliage and air temperature did change with water availability. Foliage temperatures increased as container medium dried between waterings.

For ponderosa pine, foliage temperatures began to increase relative to air temperature when lights were turned off, after 20% of the maximum attained hardiness was lost. Engelmann spruce, however, never did settle into a stable pattern. Temperatures did generally stop dropping relative to air temperature after about 20% of the maximum cold hardiness was lost. Only after about 75% of the acquired hardiness was lost did the foliage temperature increase relative to air temperature when the lights were turned off.

SUMMARY AND CONCLUSIONS

The study reported here was the first step toward determining applicability of thermal imaging systems in assessing physiological state of seedlings (for example, their status regarding dormancy or cold hardiness). As such, it must be viewed as exploratory. Because the study was opportunistic in the sense that it was added on to another, more intensive study designed to answer different questions, the amount of manipulation was limited. For this reason, and because of restrictions on the randomness of seedling selection (seedlings were

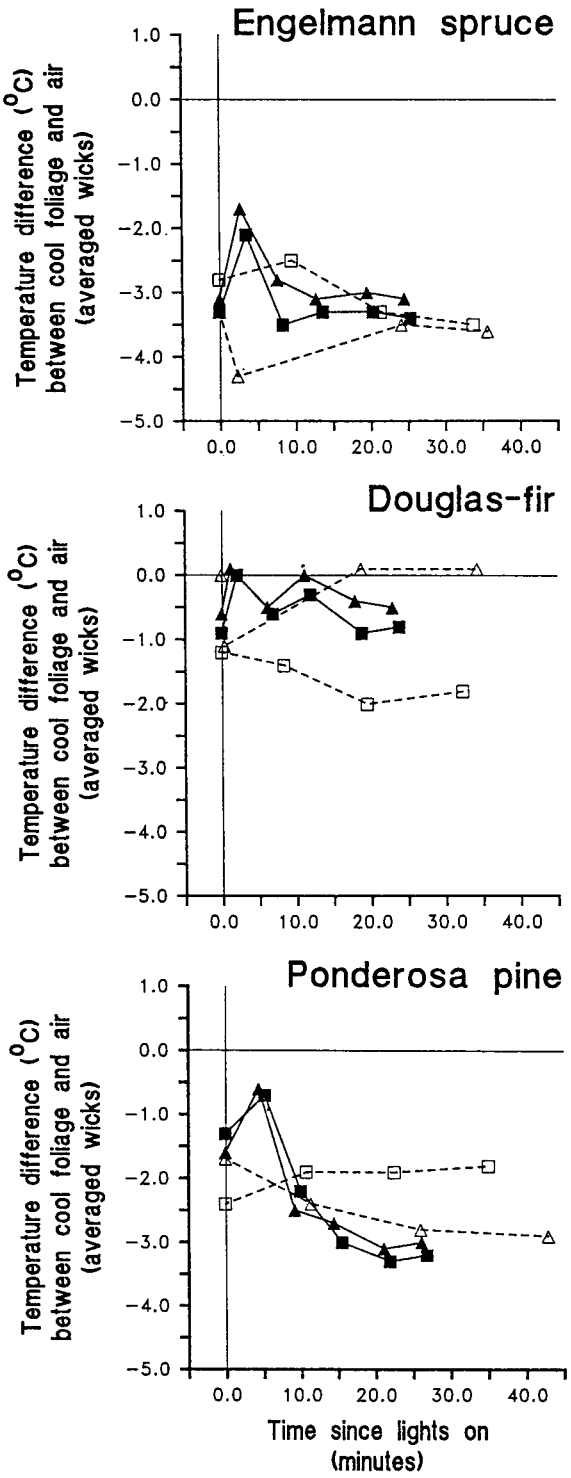

Figure 2.--Temperature response of cool regions on the seedlings during the first hour after turning lights on. Data are average temperatures of seedlings 1-4 at 3 days (open triangles) and 29 days (shaded triangles) and of seedlings 25-28 at 3 days (open boxes) and 22 days (shaded boxes) after removal from stage 3 hardening conditions.

in books of four seedlings each, were limited in number of books, and were measured in the same growth chamber) statistical analysis was limited to description of averages only. Notwithstanding, several responses were observed that could be important to further research:

• Confirmation of the observed change in temperature response with relative degree of cold hardiness during the dehardening period, and demonstration of similar changes during the hardening process, could be the basis of a useful tool.

• The observation that changes in water availability do not change the initial temperature response pattern, but only the actual temperatures achieved, must be carefully verified. If true, a series of potential obstacles to the operational use of thermography (e.g. differing soil water potential, low soil temperatures) are reduced or removed.

• Seedling response to the transition from light to dark is potentially more useful to monitor than is the transition from dark to light.

o The time of most useful response appears to be in the first 20 minutes after a change in light.

• Foliage on a seedling is not all the same temperature, and the cool areas appear to be more useful in defining response patterns than is the general temperature of the seedling.

• Engelmann spruce foliage temperatures were surprisingly low relative to air temperature and dropped as hardiness developed. This observation suggests that stomatal conductance could be high in the winter, a situation similar to coastal Douglas-fir but unexpected in a high elevation continental species.

This study has clearly identified some significant areas for further research and suggests that much could be learned about seedling physiology using thermography. There are tantalizing hints of approaches for use of the method in tracking dormancy or cold hardiness. For example, if the patterns of temperature change following removal of light are verified to be related to cold hardiness state, then an operational test might be developed that would be completed in less than 30 minutes rather than days or even weeks.

For future studies, given the timing of root growth capacity and development of cold hardiness described by Tinus et al. and Burr et al. (these proceedings), it would be desirable to schedule thermographic measurements during

the time the seedling physiology is changing rather than at times when growth chamber conditions change.

However, thermography cannot be suggested as a useful tool until some of the responses noted in the study are verified and understood.

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