

INFRARED THERMOGRAPHY FOR ASSESSING SEEDLING CONDITION-  
RATIONALE AND PRELIMINARY OBSERVATIONS

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ABSTRACT--Forest managers need quick, nondestructive tests to estimate degree of dormancy and other aspects of the physiological condition of tree seedlings. We are investigating the potential of state-of-the-art thermography for this purpose. The study is based on the possibility that plant temperature is related to important physiological attributes of seedlings in diagnostically useful ways: (1) Leaf temperature is affected by stomatal conductance, which may be influenced by dormancy status; and (2) temperature of buds, root tips, and seeds may be measurably affected by rate of metabolic activity. Preliminary observations for several coniferous species and seed sources suggest that seedling temperature varies significantly in relation to length of time since beginning of emergence from the last stage of dormancy. But efforts to date to use thermography for measuring differences in metabolic activity of conifer buds, roots, and seeds have been unsuccessful.

#### 10.1 INTRODUCTION

For successful plantation establishment, seedlings must be in proper condition to survive the shock of outplanting and to start new root and shoot growth rapidly. Estimates of seedling quality pertinent to survival and growth potential are needed at all steps in the process of producing and using planting stock--from nursery bed to planting site (and desirably even to plantation establishment). Morphological characteristics of roots and shoots have long been important criteria used for assessing seedling quality. In recent years, however, regeneration foresters, nursery managers, and researchers have become more aware that physiological attributes not identifiable visually are critical to seedling performance potential. The various methods of evaluating seedling quality are described elsewhere in this volume and in an excellent review by Ritchie (1984).

"Performance" attributes of planting stock, such as root growth capacity (or potential), frost hardiness, and stress resistance, are based on whole seedling tests that integrate

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the functions of many seedling systems (Ritchie 1984). Consequently, these attributes often correlate well with seedling performance potential. Since performance attributes can change dramatically and unpredictably during seedling storage, however, their value as predictors of seedling performance is questionable unless tests are performed at the time of planting. But by that time, results from these relatively time-consuming tests may come too late to permit meaningful management responses.

An important descriptor of seedling condition, generally related to the performance attributes, is dormancy status. The ability to identify the onset and intensity of dormancy is needed to (a) define nursery cultural regimes to control the initiation of dormancy; (b) determine safe lifting windows (Jenkinson 1984) and permissible storage temperatures and durations; and (c) assess the significance of cooler failures, improper handling, delayed planting, or other problems. Unfortunately, no rapid method of measuring dormancy status is presently available (Ritchie 1984).

The need exists, therefore, for quick, nondestructive tests to estimate seedling dormancy and other aspects of physiological condition. Therefore, we began an effort to explore the potential of state-of-the-art thermal imaging equipment for assessing seedling condition. We used an imaging radiometer that can display a television-like thermal image of a scene and permit quantitative temperature measurements within the scene.

This paper describes the rationale for investigating infrared thermography as a possible tool for assessing seedling condition, and offers preliminary observations to date.

## 10.2 RATIONALE

Thermal sensing devices measure apparent temperature of surfaces--in this case, leaves or other plant parts. Among the various factors that influence plant temperature, two may be related to physiological condition of the plant in diagnostically useful ways. Together they constitute the basis for hypothesizing that infrared thermography can be an effective tool for assessing seedling condition: (1) leaf (and possibly bud) temperature as affected by stomatal conductance and transpiration rate, and (2) bud, root, and seed temperature as affected by rate of metabolic activity. These two factors are, of course, very different in nature, and the magnitude of energy transfer in the two cases differs considerably as well. For these reasons, they will be discussed in separate sections.

### 10.2.1 Stomatal Conductance

Leaf temperature is a complex function of the various components of the leaf energy balance,

which involves both plant and environmental factors (Gates 1980, Knoerr and Gay 1965). For a given leaf, the dominant physiological factor influencing the energy balance (and thus leaf temperature) is stomatal conductance, because of its effect on transpirational cooling. Although the physiological significance of transpirational cooling is debatable, the fact is that under most sets of environmental conditions, a leaf with high stomatal conductance (open stomata, leading to a higher transpiration rate) is measurably cooler than an otherwise identical leaf with low stomatal conductance (closed stomata, leading to a lower transpiration rate).

Stomatal conductance is strongly affected by leaf water stress. Therefore, leaf temperature can be used to estimate plant water stress (Jackson et al. 1981, Puritch 1981) or other plant attributes dependent on water stress (Agricultural Age 1982, Alger et al. 1980, Walker and Hatfield 1983). Our own observations tend to confirm the existence of relationships between leaf temperature and water stress for seedlings of several California conifer species. If these relationships can be made functionally usable, they may provide a means to evaluate the water status of seedlings much more quickly than is possible by using the pressure bomb.

Of potentially greater significance is limited evidence that stomatal conductance varies with phenological state of a plant (Hinckley et al. 1978, Jarvis and Mansfield 1981) and, in at least some conifers, between active and dormant seasons (Christersson 1972, Kozlowski 1943, Parker 1963, Weaver and Mogensen 1919, Zelawski and Kucharska 1967). This evidence suggests that, in comparison with their behavior during periods of active growth, stomata during the dormant season tend to be more nearly closed (lower conductance) and to respond less to environmental factors, such as light, that normally influence stomatal behavior. To the extent that such changes in conductance and associated leaf temperature are diagnostic, thermography could be developed into a useful tool for assessing seedling condition.

Assuming that conductance is in fact found to be an indicator of dormancy status for the conifer species of interest, several obstacles must be overcome before thermography can become an operational tool. First, the apparent temperature of a seedling as determined with thermography depends, among other conditions, upon seedling-scanner geometry. If the temperature of a single conifer needle is to be measured accurately, the width of the needle must occupy at least a certain critical proportion of the scanner field of view. Viewing the needle at a sufficient relative size to ensure accurate measurements precludes the broader view that might be desirable for many applications. Possible practical solutions may include correcting for background temperature or ensuring that most of the background consists of seedling foliage, such as in oblique views of nursery beds.

Second, as indicated earlier, temperature of a needle depends upon a number of plant and environmental variables that define the energy balance of the needle. Stomatal conductance is only one of those variables. Therefore, in order to isolate the effects of conductance, it will be necessary to account for, or "normalize," the other variables that affect the observed temperature. (The only alternative may be to restrict observations to test chambers with rigidly-controlled environmental conditions). Further, prospective tests will benefit by defining those conditions that enhance the sensitivity of temperature changes to changes in conductance--i.e., that tighten the linkage between conductance and temperature.

Third, the component of stomatal conductance (or change in conductance) attributable to dormancy status or other physiological condition to be evaluated must be separated from that affected by other factors such as light, humidity, and (unless it is the subject of interest) plant water stress. In order to detect temperature differences associated with, say, intensity of dormancy, we would want conductance to be able to vary from low to high as a function of dormancy. Therefore, we probably would want to avoid such test conditions as darkness or high water stress that would promote stomatal closure and thus preclude high conductance regardless of dormancy status. The possible complicating effects on conductance of extraneous factors might be more than compensated by opportunities to include these factors in test procedures. For example, the response of stomatal conductance (as estimated by leaf temperature) to changing light intensity might have more diagnostic value in assessing dormancy status than conductance estimated under static conditions involving a single light intensity.

#### 10.2.2 Metabolic Activity

Differences or changes in some aspects of seedling physiological condition, including dormancy status, may be expressed by differences in rates of respiration or other metabolic processes. Presumably, such differences would become apparent first in meristematic tissues such as buds and root tips.

Similarly, the metabolic activity of seeds accelerates as they progress from deep dormancy through stratification to germination. Conceivably, seed-to-seed variations in metabolic rates during stratification could foretell related variations in germination and subsequent seedling vigor. (Seeds obviously are not seedlings. However, we mention them here briefly because of the clear relationship between seed quality and seedling quality.)

It seems possible to us to use thermography to detect temperature differences associated with differences in metabolic activity. Heat is a byproduct of metabolic activity and can, in fact, be measured in calorimeters. Under certain conditions, metabolic heat production

also can be manifested in a measurable temperature increase, as evidenced by the commonly-observed temperature gradient within a bag of seedlings in cold storage.

### 10.3 METHODS AND PRELIMINARY OBSERVATIONS

Our work to date has consisted of preliminary observations rather than carefully designed and controlled experiments because we have lacked some of the resources for controlling and monitoring environmental conditions and measuring seedling characteristics that we consider necessary for rigorous studies. In addition, we concluded that a relatively cursory look at several possible approaches and types of plant material would help us determine the more promising areas for subsequent detailed, specific studies.

#### 10.3.1 Stomatal Conductance

If stomatal behavior were found to be correlated usefully with dormancy intensity for at least some western conifers, the implications for potential use of thermography in assessing seedling dormancy status could be significant. We decided, therefore, that relationships among stomatal conductance, seedling temperature, and dormancy would be a productive area for concentrating our initial efforts. The nomenclature of dormancy used here follows that in Lavender (1984).

Most of our observations to date have been associated with seedlings emerging from postdormancy. Currently we are monitoring similar sets of seedlings undergoing dormancy initiation and movement into deep dormancy.

##### 10.3.1.1 Emergence from postdormancy

Methods.--In early June 1984, we obtained barefoot planting stock from the USDA Forest Service nursery at Placerville, California. The stock consisted of 11 California seed sources comprising six species--ponderosa pine, sugar pine, lodgepole pine, Douglas-fir, white fir, and red fir. For all species except lodgepole pine, we used two seed sources representing a range in latitude, elevation, or both. The northern, low elevation ponderosa pine source was 1-0 stock; all other sources were 2-0. The seedlings were transported to Redding, California, and placed in a cooler facility (a temporarily converted growth chamber), which was maintained at a nominal temperature of 1°C. Packing material in a number of the bags did freeze during storage, however.

After several days of storage, seedlings were removed, thawed carefully where necessary, and separated into comparable groups based on morphological characteristics. For each seed source, seedlings were divided first into two major groups--one to be used beginning immediately for monitoring emergence from postdormancy, the other to be used later for following dormancy initiation. Each major

group was further divided into three subgroups to permit a time sequence of changing environmental regimes. For "spring" seedlings, the subgroups would be removed from cold storage and planted (emergence from postdormancy) at 2-week intervals. The "fall" seedlings would be moved at 2-week intervals in late summer into an environmental regime for promoting dormancy initiation.

All fall seedlings were planted immediately, using a 1:1 vermiculite-peat moss potting mixture, and placed in the greenhouse. For each seed source and subgroup, four seedlings were planted in individual half-gallon milk cartons perforated to permit adequate drainage, and eight were planted in a redwood box. Seedlings in individual containers were intended to permit easier individual manipulation and observations, including gravimetric measurements of transpiration. The other seedlings were available for other uses, including destructive sampling.

At the same time, and in the same manner, one of the three subgroups of "spring" seedlings from each seed source was planted. The other subgroups were returned to cold storage. The second subgroup was planted 14 days later, the third subgroup, 28 days later. These subgroups are referred to hereafter as "0-day seedlings", "14-day seedlings", and "28-day seedlings". After seedlings were planted and moved into the greenhouse, they were kept adequately watered and fertilized.

Two days after the first sets of seedlings were planted, and while the 14-day and 28-day seedlings were still in cold storage, the compressor on the cooler/growth chamber failed. By the time the failure was discovered 14 hours later, in-bag temperatures had risen to about 26°C. Seedlings were immediately moved into refrigerators, but in-bag temperatures did not return to the vicinity of 1°C until 24 to 30 hours later. Despite the failure, we continued with the study. The implications are discussed later.

Two days after the 28-day seedlings had been planted and moved into the greenhouse, we began several short experiments to explore relationships between length of time since commencement of emergence from postdormancy and seedling temperature and transpiration rate. Direct measurements of stomatal conductance would have been desirable but were impractical without a diffusion porometer. Since determining comparative transpiration rates was sufficient at this early stage of the research, we simply estimated environmental conditions and exposed the different subgroups of seedlings to the same conditions for the same periods of time.

Our general hypothesis in these experiments was that the three subgroups of seedlings should encompass a "gradient" in degree of dormancy, ranging from 28-day seedlings (most recently emerged from cold storage, thus presumably most nearly dormant) to 0-day seedlings; and that a corresponding gradient in stomatal behavior and

seedling temperature should be evident, with the stomates of more dormant seedlings tending to be more nearly closed and to respond less to environmental stimuli.

The first of two experiments reported here was designed to measure transpiration rates of the test seedlings during light and dark periods. Ten of the 11 seed sources were included (one red fir source was not used because of excessive mortality). Two seedlings in each subgroup were selected to be as nearly uniform as possible. Individual seedling containers were well watered, drained, and then sealed in plastic bags so that measured weight loss would consist solely of seedling transpiration. Two dead seedlings in containers were prepared similarly as controls to correct for any slight evaporational losses.

After containers were prepared, we removed all new, succulent foliage (present on nearly all of the 0-day seedlings and some of the 14-day seedlings) in order to avoid any confounding effect of different ages of needles. Seedlings then were weighed and placed outside to begin the first light period. Conditions (full sunlight, approximately 40°C, 20 to 30% RH, 3 to 5 mph windspeed) favored rapid transpiration. Seedlings were reweighed at the end of a known interval averaging about 2 1/2 hours and placed in a growth chamber regime under these conditions: no lights, 25°C, 70% RH, and slight air movement. They were moved the next day back outside, under conditions of somewhat lower transpirational demand, to complete the final light period. Transpiration rates for each seedling during each period were calculated in grams of water loss per seedling per hour. (Some error certainly is introduced by failing to express transpiration on the basis of needle area or mass. However, the seedlings representing a given seed source were relatively uniform, and we had neither the equipment to estimate leaf area on intact seedlings nor the desire to expend the seedlings in destructive sampling.)

The three spring subgroups were necessarily planted at different times because we started with bareroot stock in cold storage. The capacity of the root system to absorb water rapidly--dependent to a large extent on new root growth--thus developed at different times. In comparing transpiration rates of the different subgroups, therefore, we were faced with a possible confounding factor: Any observed reduction in light period transpiration for, say, newly-planted 28-day seedlings might be partly attributable to an inadequately functioning root system, which could lead to development of water stress and stomatal closure. To get some indication about the probability of such an effect, we measured plant moisture stress on other seedlings of each subgroup subjected to the same environmental regimes just described. We found no significant differences in "pre-dawn" (emerging from dark period, low stress environment) moisture stress, but at mid-day, 28-day seedlings showed significantly higher

moisture stress (lower xylem pressure potential)--mostly 20 to 30 bars--than did 0-day seedlings--mostly less than 10 bars.

A second experiment was conducted in which this confounding effect was avoided. Six seed sources were used--one each of ponderosa pine, Douglas-fir, white fir, and red fir, and two of sugar pine. Well-watered seedlings were kept overnight in the growth chamber in the dark at 20°C and 70% RH to bring them to a relatively uniform, low moisture stress. They were then illuminated for 1 to 2 hours, under continuing conditions of low transpiration demand, to promote stomatal opening.

For each seed source, six similar seedlings--two per subgroup--were selected for measurement. New foliage was clipped, and the cut ends were sealed with paraffin film. Each seedling was severed at the root collar, the cut end sealed, and the shoot immediately weighed. (Severed shoots were used in this experiment to permit the more precise weighings needed during a relatively short measurement period.) The six severed shoots were then set up on a laboratory table for videotaping of the thermal image with the imaging radiometer. Immediately thereafter seedlings were reweighed and laid out to continue dehydrating. Laboratory conditions were approximately 28°C, 30 to 40% RH, negligible windspeed, and fluorescent lighting near that of a typical office. Seedlings of each seed source subsequently were reweighed at least two more times to establish initial patterns of transpiration decline with time. Finally, needles from each seedling were removed and oven-dried to permit expression of transpiration rates in terms of dry weight of foliage.

Results and discussion. The transpiration rate patterns in the first experiment, which encompassed a light-dark-light sequence, were reasonably similar in ponderosa pine, sugar pine, Douglas-fir, and white fir (fig. 1). Although substantial variation in rates often exists between the two seedlings of a subgroup, the trends are apparent: Light period transpiration rates were high for 0-day seedlings, low for 28-day seedlings, and usually intermediate for 14-day seedlings. In contrast, dark period rates were consistently low for all three subgroups. The generally lower rates during the second light period are, we believe, attributable to lower average transpiration demand during this period. The transpiration patterns for species and seed sources not illustrated are similar to those described above.

The pattern for red fir was consistent with the other species during the dark period (fig. 1). Light period rates, however, showed high variability within subgroups and no consistent differences among subgroups. This could be a characteristic of this species or seed source, or a fluke associated with this particular small sample of seedlings. It is an interesting corollary observation, however, that the red fir sources were, as explained

later, the only ones in which we subsequently observed significant mortality. To what extent this may have been related to the cooler failure we do not know. In any case, whatever stresses or physiological alterations led to the mortality might also have been involved in the atypical transpiration patterns.

During light periods, seedlings that had been taken from cold storage and planted earlier appear to have greater stomatal openings--hence higher transpiration rates (fig. 1). Stomata of 28-day seedlings apparently did not open much, if any, in response to light. During the dark, all seedlings transpired at low rates, suggesting closed stomata for all subgroups. These observations are consistent with our hypothesis concerning dormancy status and stomatal conductance. They also are consistent with results reported by Christersson (1972) in a related study using Scots pine and Norway spruce.

This experiment involved the confounding factor of differing degrees of root system establishment associated with time of planting. Therefore, we cannot say how much of the reduced stomatal conductance (as evidenced by reduced transpiration) of 28-day seedlings in the light was related directly to dormancy, and how much was caused by high water stress induced by limited absorption capacity of roots. Since results of the first experiment are reasonably consistent with those of the second (unaffected by this confounding effect), however, differences in dormancy status may in fact have accounted for a major portion of the observed patterns.

Transpiration rates for detached shoots were measured in the second experiment. High initial rates declined rapidly, reflecting decreasing stomatal conductance of seedlings as water stress developed. Seedlings with lower initial rates--lower stomatal conductance--maintained a more nearly constant rate with time, as expected (fig. 2). Rates of all seedlings converged toward those associated with closed stomata.

The initial rates--the first points on the drying curves--should correspond closely with those to be expected from comparable intact seedlings. Furthermore, they represent the average transpiration rate over the period during which temperature was measured. Higher transpiration rates lead to lower leaf temperatures. To facilitate direct comparison of temperatures and initial transpiration rates, therefore, we inverted the temperature scale in figure 2.

Two significant points were evident for all the species illustrated: (1) Although variations certainly exist, initial transpiration rate was closely related to foliage temperature. While not surprising, this relationship bodes well for potential applications of thermography for assessing seedling condition. (2) The 28-day seedlings (presumably more nearly dormant) were consistently warmer, and had a consistently lower initial transpiration rate, than the

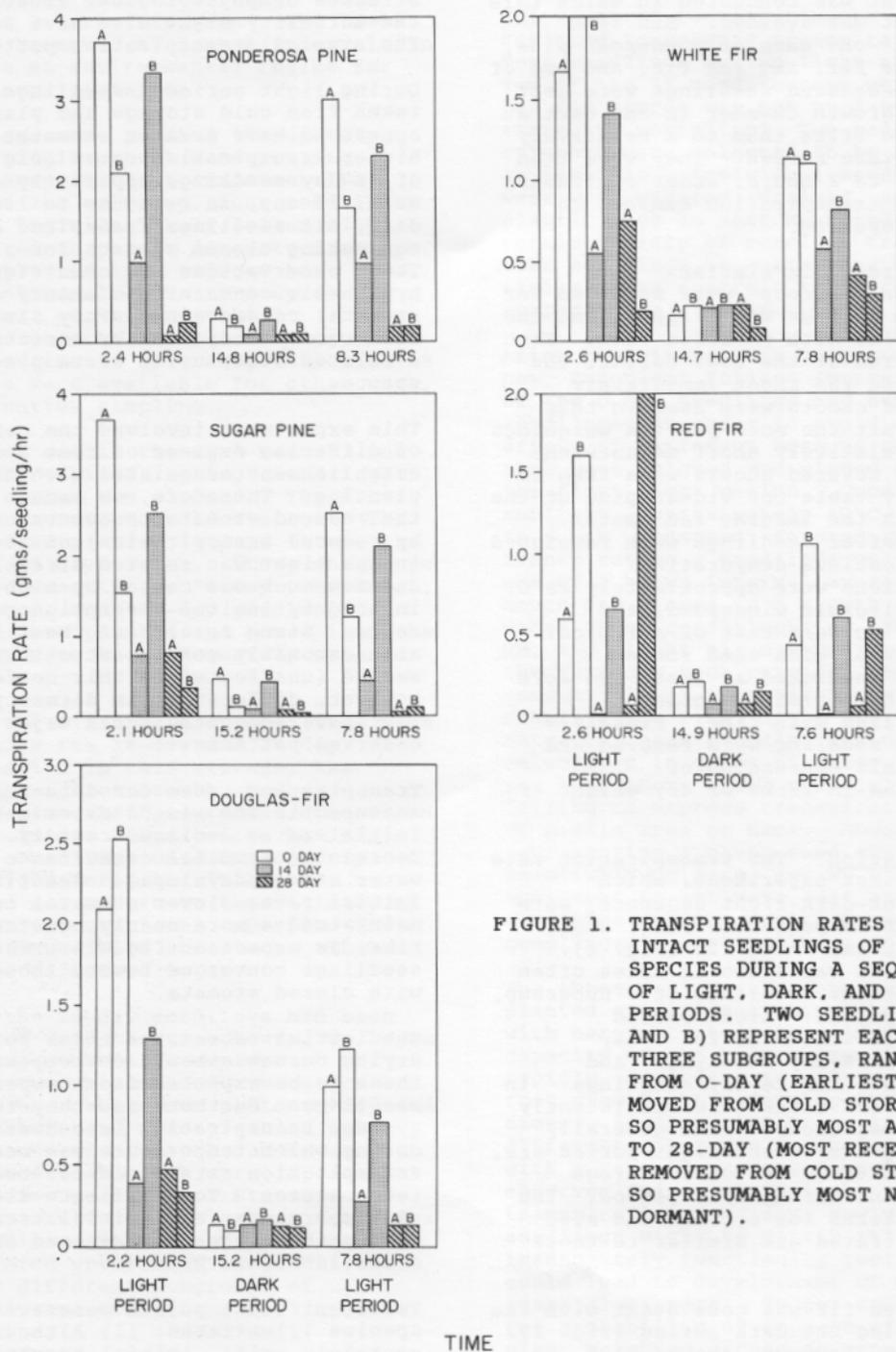
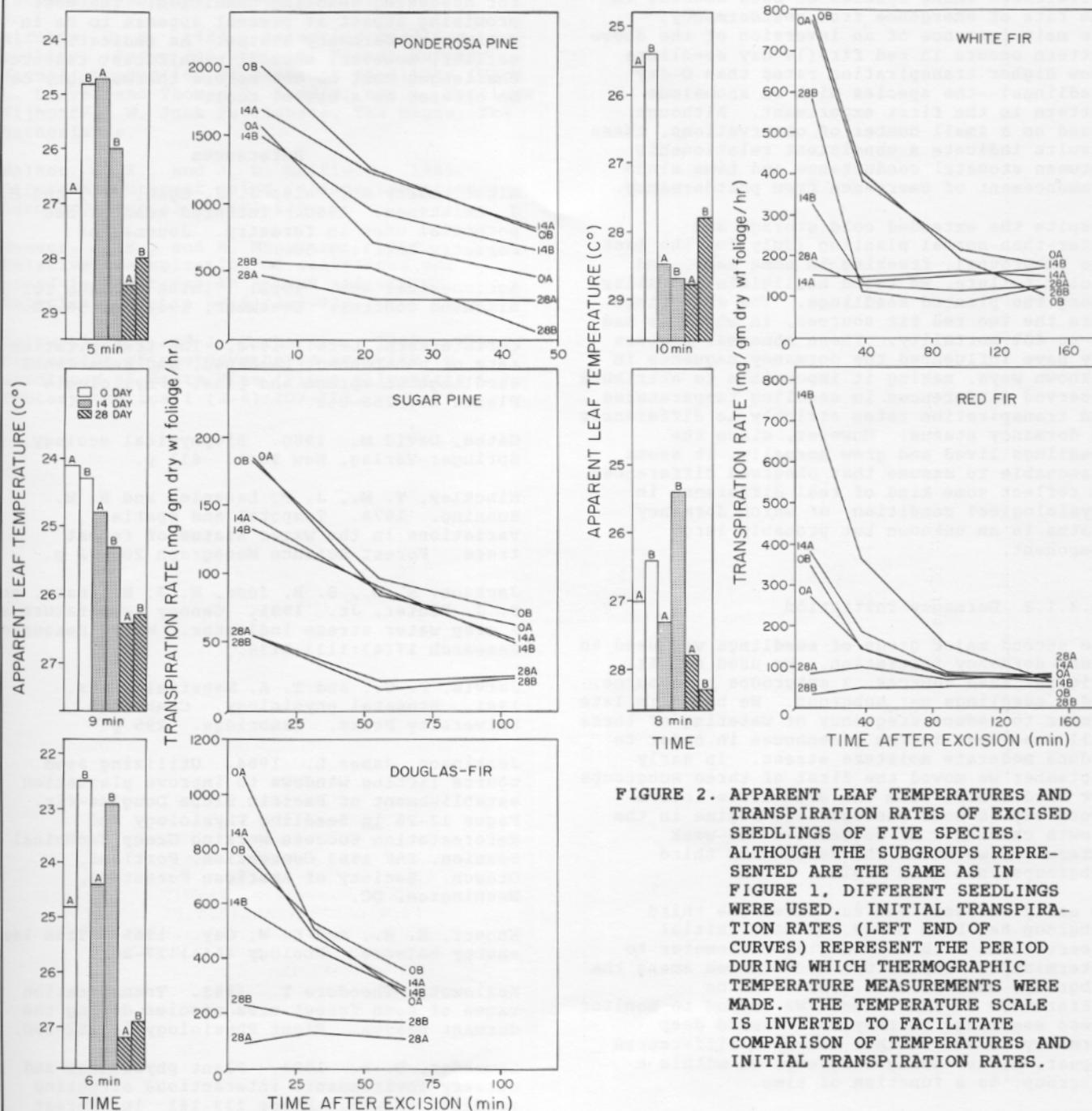


FIGURE 1. TRANSPIRATION RATES OF INTACT SEEDLINGS OF FIVE SPECIES DURING A SEQUENCE OF LIGHT, DARK, AND LIGHT PERIODS. TWO SEEDLINGS (A AND B) REPRESENT EACH OF THREE SUBGROUPS, RANGING FROM 0-DAY (EARLIEST REMOVED FROM COLD STORAGE, SO PRESUMABLY MOST ACTIVE) TO 28-DAY (MOST RECENTLY REMOVED FROM COLD STORAGE, SO PRESUMABLY MOST NEARLY DORMANT).

TIME



**FIGURE 2.** APPARENT LEAF TEMPERATURES AND TRANSPIRATION RATES OF EXCISED SEEDLINGS OF FIVE SPECIES. ALTHOUGH THE SUBGROUPS REPRESENTED ARE THE SAME AS IN FIGURE 1, DIFFERENT SEEDLINGS WERE USED. INITIAL TRANSPIRATION RATES (LEFT END OF CURVES) REPRESENT THE PERIOD DURING WHICH THERMOGRAPHIC TEMPERATURE MEASUREMENTS WERE MADE. THE TEMPERATURE SCALE IS INVERTED TO FACILITATE COMPARISON OF TEMPERATURES AND INITIAL TRANSPIRATION RATES.

0-day seedlings. The 14-day seedlings tended to range near either the 0-day or 28-day seedlings, or somewhere between. The relative position of the 14-day seedlings may reflect differences among species or seed sources in the rate of emergence from postdormancy. The main instance of an inversion of the above pattern occurs in red fir (14-day seedlings show higher transpiration rates than 0-day seedlings)--the species with an anomalous pattern in the first experiment. Although based on a small number of observations, these results indicate a consistent relationship between stomatal conductance and time since commencement of emergence from postdormancy.

Despite the extended cold storage and later-than-normal planting (July for the last two subgroups), freezing in some bags, and cooler failure, we found negligible mortality among the planted seedlings. The exceptions were the two red fir sources, in which we had 30 to 40% mortality. These abnormal factors may have influenced the dormancy sequence in unknown ways, making it impossible to attribute observed differences in seedling temperatures and transpiration rates strictly to differences in dormancy status. However, since the seedlings lived and grew normally, it seems reasonable to assume that observed differences do reflect some kind of real difference in physiological condition, of which dormancy status is an unknown but probably large component.

#### 10.3.1.2 Dormancy initiation

The second major group of seedlings was used to study dormancy initiation. We used our 11 original seed sources, 3 subgroups per source, and 4 seedlings per subgroup. We began in late summer to reduce frequency of watering of these fall seedlings in the greenhouse in order to induce moderate moisture stress. In early September we moved the first of three subgroups per seed source from the greenhouse into a short day (8-hour photoperiod) regime in the growth chamber. At successive two-week intervals, we moved the second and third subgroups into this regime.

In early October, one day after the third subgroup had been moved, we made initial observations with the imaging radiometer to determine if temperature differences among the subgroups were evident. We found no differences at that time. We intend to monitor these seedlings as they move toward deep dormancy to see whether and when differences appear, either among subgroups or within a subgroup, as a function of time.

#### 10.3.2 Metabolic Activity

We conducted several small trials in an attempt to observe temperature differences associated with differences in metabolic activity of both seeds and seedlings. None has proven successful to date.

## 10.4 CONCLUSIONS

On the basis of our limited work to date, infrared thermography shows potential as a tool for assessing seedling condition. The most promising aspect at present appears to be in estimating dormancy status. As indicated earlier, however, several significant research challenges must be met before thermography can be offered as a useful tool.

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