SEASONAL VARIATION IN METABOLIC RATE AND ITS CORRELATION TO HALF-SIB FAMILY PERFORMANCE IN LOBLOLLY PINE

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Abstract -- Measurement of metabolic rate by heat conductance calorimetry (HCC) has been previously shown to predict long-term growth performance in larch and coastal redwood. In order to demonstrate the validity of this technique as a selection tool in loblolly pine, the metabolic rate of six seed orchard clones of known height performance level (PL) was measured throughout the growing season using a differential scanning calorimeter operating in the isothermal mode. Metabolic rates varied during the 1990 growing season, with rates highest just prior to the spring growth flush and lowest during high summer. Metabolic rates began to increase in mid-August and rose to levels comparable to those measured in early spring. The late season increase in metabolic rate was coincident with (1) the initiation and development of female strobili primordia and (2) and additional cycle of shoot growth with the relief from the droughty conditions that persisted through most of the spring and summer of 1990. When metabolic rates were measured right before the first flush of growth in the spring, the rate was found to correctly rank four of five clones sampled and only the order of the two lowest height PL clones was switched. Particularly noteworthy in this ranking was that the height PL data was for the progeny of the seed orchard clones while the metabolic rate measurements were made on the parents. Therefore it appears that metabolic rate measurements can be used to identify good seed orchard clones, as well as outstanding individuals for vegetative propagation. The dependence of metabolic rate on the environment, the developmental status of vegetative shoots and the differences in clone phenology with respect to shoot growth cycles suggests that genetic comparisons should only be made when trees are in the same physiological condition.

Keywords: Pinus taeda L., heat conductance calorimetry, metabolic rate, genetic selection, height performance level.

INTRODUCTION

Inasmuch as the rotation age of loblolly pine (Pinus taeda L.) can be several decades long, advanced generation selection has been focused on evaluating juvenile performance characteristics

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in progeny tests. Selection typically occurs 4-8 years after progeny test establishment. Several early selection experiments have attempted to reduce the selection age further with varying results. Some of the more promising experiments in early selection have examined iterative growth processes such as shoot growth cycles (Bridgwater, 1990), and nutritional considerations (Li et al, 1989).

The contributions of nutritional and physiological parameters to growth suggest that techniques for early selection may be developed that are independent of growth measurements. Such "laboratory" techniques would concentrate on processes intrinsic to the cells or tissues in a tree and would not require measurements of field performance. It has been forwarded (Hansen et al., 1989) that the obvious candidates for laboratory tests of intrinsic growth processes (photosynthetic rates and carbon dioxide uptake rates) have not been successful predictors of future performance because these processes are seldom rate limiting to growth. On the other hand, if the rate limiting step to growth is the incorporation of fixed carbon dioxide into biomass, then a measure of the metabolic rate should be predictive of the growth potential.

Measurement of metabolic rates (by HCC) in larch (Hansen et al., 1989) and coastal redwood (Hart Scientific Co., 1989) have been previously correlated to long-term growth potential in these species. In order to investigate the application of this technique to loblolly pine, metabolic rates were measured throughout the season on seed orchard clones of known height PLs.

MATERIALS AND METHODS

Plant Material

Study trees were selected from a second-generation loblolly pine seed orchard established ca. 1976 near Savannah, GA. Clones studied represented selections of known height PL. Height PL was determined from open-pollinated progeny tests using the methods of Hatcher et al (1981). For metabolic rate measurements recorded throughout the season, three clones were chosen as "poor" performers (height PL = 25 ± 6) and three as "good" performers (height PL = 79 ± 2). Shoot tips were collected from the same ramet in each clone, and all ramets were located in the same block of the orchard. Shoot tips were collected from the upper 25% of the crown. Care was taken to avoid shoots that were damaged by insects or disease. Shoot tips were wrapped in moist paper towels and shipped on ice overnight for HCC. Shoot tips could be stored at 4°C for up to 12 days prior to metabolic rate measurements.

In an experiment aimed at investigating the effect of shoot development status on metabolic rate, fourteen seed orchard clones encompassing the entire range of performance level were analyzed in mid-May, 1990. At the time of sampling, shoot tips were placed into four categories with respect to the degree of shoot elongation. The four categories were: (1) a dormant, or "winter" type bud, (2) a winter type bud that appeared to be on the verge of flushing, (3) shoot tips in which the needle fascicles had elongated less than 15 mm ("pinfeather" stage) and (4) shoot tips in which needle fascicles had elongated more than 15 mm.

Heat Conductance Calorimetry

HCC was conducted using a Du Pont model 910 Differential Scanning Calorimeter operating in the isothermal (27.0°C) mode. In order to accommodate a loblolly pine shoot tip, custom stainless steel sample holders were constructed by Chatam Precision (Union, NJ). The cylindrical sample holders had a threaded lid that was fitted with a neoprene rubber gasket. Using this sample holder,

shoot tips approximately 7 mm in length, with a fresh weight on the order of 40-70 mg, were analyzed.

All metabolic rate determinations were performed at least in triplicate, and each determination required about 20 min to reach equilibrium. Since it often took several days day to perform HCC analysis for all the samples from a particular collection date, analysis of the first clones sampled was repeated to confirm that no sample degradation was occurring over the period of analysis. All samples were run double-blind; neither the HCC operator or the person preparing the sample knew the identity of the samples under analysis.

RESULTS AND DISCUSSION

Figure 1 shows a typical time course for metabolic rate determinations. The direction of approach to equilibrium (the curved portion of Figure 1) is from negative heat fluxes reflecting the fact that since the metabolic rate was measured at temperatures greater than ambient, heat had to first flow into the sample to return to the measurement temperature. The absolute difference in the two flat portions of the Figure 1 (the baseline **is on** the left), expressed in microwatts (µW), is the heat producer] by the sample and is taken as the metabolic rate.

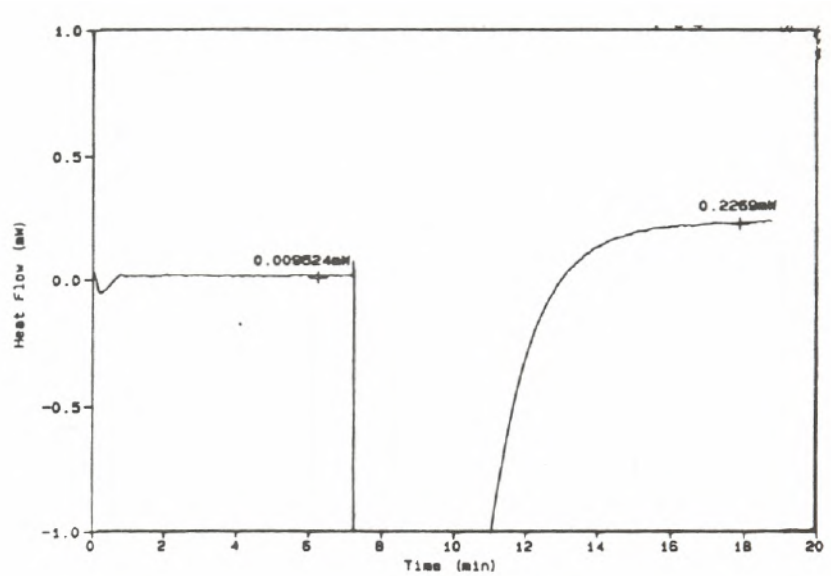

Figure 1. Sample data plot for calorimetric measurement of metabolic rate of loblolly pine shoot tips (see text for explanation).

Metabolic rates reported here for loblolly pine averaged about 5-10 µW/mg throughout the growing season are in agreement with values reported for other conifers. For example, metabolic rates of balsam fir and larch recorded in the summer are both reported to be about 15 µW/mg (Hansen et al., 1989), and those for coastal redwood about 1.0 µW/mg (Hart Scientific Co., 1989).

Figure 2 shows the variation in metabolic rate for the high and low height PL clones for a 194 day period beginning March 6, 1990. Note that at no time throughout this period was the metabolic rate of the high height PL clones significantly ($p = 0.05$) greater than the low height PL clones. However, statistically significant differences were observed in the metabolic rates for different sampling dates. Metabolic rate started out highest just prior to the spring growth flush, but diminished after that until it reached a minimum in mid-July. Metabolic rate began to increase

in August and reached values comparable to those observed in the spring. Rates then remained high throughout the fall, but dropped dramatically in late-October to levels below 1.0μW/mg (data not shown). The increase in metabolic rate in August was coincident with two observations: (1) relief from the droughty conditions that had persisted since March, and (2) the differentiation of female cone primordia that were observed in shoot tip sections by September (Greenwood, 1980).

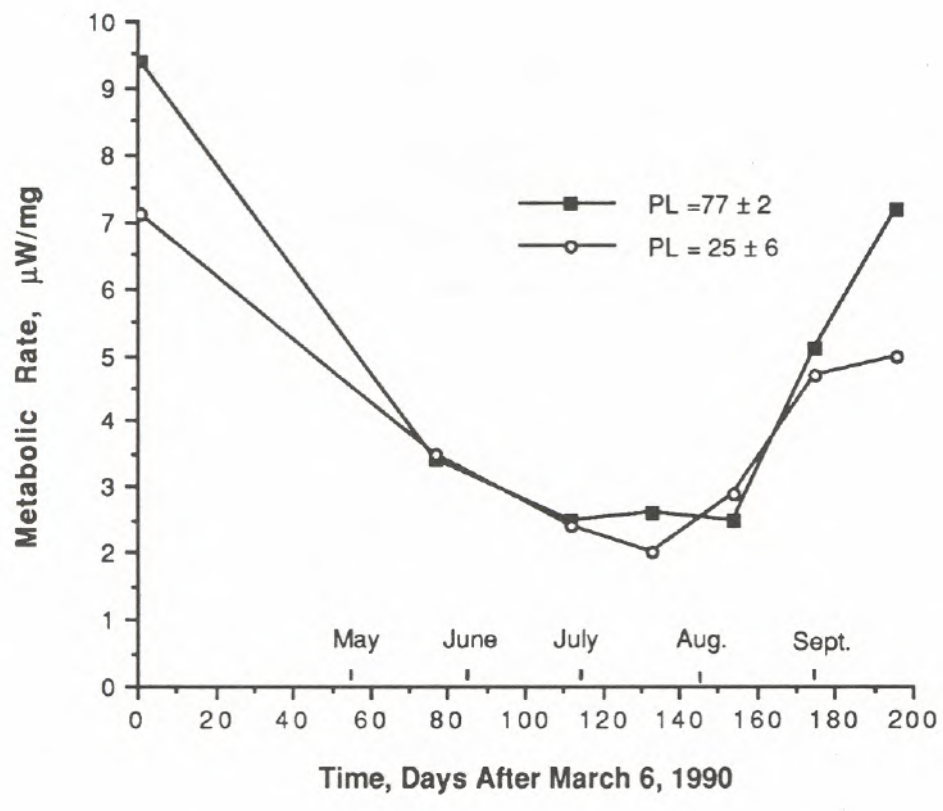

Figure 2. Seasonal variation in metabolic rate of loblolly pine for high and low height PL seed orchard clones. (For a given date, differences between high and low height PL clones were not significant as determined by independent t-test. Before May and after September, means were significantly different by paired t-test.)

Indictment of the hot, dry conditions that persisted near Savannah during the 1990 growing season as the reason for the decline in metabolic rate is evident in Table 1. In 1990, during the three month period (March-June) rainfall totalled only 3.77" and the metabolic rate dropped significantly for both good and poor performing genotypes. In 1991, abundant precipitation was recorded at the seed orchard and no significant reduction in metabolic rate has been observed from March to June for both good and poor performing clones. From what is known of meristematic activity in pine shoot apices, it would be expected that metabolic rates would rise through spring and peak in summer as long as conditions were favorable for growth (Cannell et al., 1976). These

observations suggest that prior to correlating metabolic rates to growth potential, consideration should be given to environmental factors that can influence metabolic rate.

Table *I.* Comparison of metabolic rates of loblolly pine seed orchard clones in two consecutive years of high and low rainfall (means followed by common letters are not significantly different as determined by Tukey's HSD).

Year	Rainfall, in (Mar.-June)	Mean Height PL	Metabolic Rate, µW/mg Early Mar.	Late May
1990	3.77	77	**9.4** a	3.4 b
		25	7.1 a	3.5 b
1991	20.49	77	9.1 a	**8.4** a
		25	8.0 a	9.1 a

In addition to possible environmental and reproductive ontological influences, differences in the extent of shoot development in each clone was demonstrated to have an effect on metabolic rate. Although all the clones examined were chosen from the same geographic region, and steps were taken to minimize environmental variation among the ramets sampled, there was considerable variation in the rate of shoot elongation between clones. Therefore, at any particular sampling time (except during dormancy), shoots could be categorized into the four previously described groups, depending on the extent of shoot elongation. Among fourteen clones sampled during mid-May as the elongation of "summer" shoots (typically the second growth flush of the season) was beginning, significant differences in metabolic rates were observed between the various shoot types shown in Table 2. Shoots in the "pinfeather" stage had the highest metabolic rates, but it is of interest to note that these clones also had the lowest height PLs. In contrast, three of the four clones that were the farthest along in the elongation of the the first summer shoot were among the best clones in the orchard, but had the lowest metabolic rate at the time of sampling. Apparently, shoot tip metabolism during shoot and fascicle elongation may not be as intense *as* during the time of bud break.

Table 2. The influence of the extent of shoot elongation (collected 5/14/90) on the metabolic rates of fourteen loblolly pine seed orchard clones (within a column, means followed by common letters are not significantly different as determined by Tukey's HSD).

Shoot Elongation Class	Clone, No.	Mean Height PL	Mean Metabolic Rate,
Dormant; between flushes	2	57 a	3.0 a
Swollen; about to flush	3	57 a	**3.9** a
"Pinfeather stage"	5	**32** b	6.4 b
Needle fascicles > 15 mm	4	65 a	1.7 a

The cosegregation of height PL and metabolic rate when shoots were classified according to their state of development may provide insight into the dynamics of shoot growth cycles and supplement early selection approaches based on shoot elongation patterns (Bridgwater, 1990). The observation that metabolic rate is a function of shoot development means that genetic comparisons should take into account variations in the extent of shoot elongation. Therefore, a single, common, sampling date for all clones in a study may not be possible.

Accurate identification of good and poor performing families is more important than the overall correlation coefficient for early selection (Caner et al., 1990). Bearing in mind that genetic comparisons should be restricted to trees that are in the same developmental point in a shoot cycle, Table 3 ranks five clones that were sampled just prior to the spring growth flush in descending order of metabolic rate. At the time of sampling in early March, these five clones still had an encased bud that had not yet begun to swell. In Table 3 it can be seen that ranking with respect to metabolic rate correctly identified the top three clones and only switched the order of the two clones with the lowest height PL. Particularly noteworthy in all these attempts to correlate performance with metabolic rate, was that while height PL is based on analysis of the progeny of seed orchard clones, metabolic rate determinations were made on the parents. Therefore it appears that metabolic rate determinations may identify good seed orchard clones, as well as outstanding individuals for vegetative propagation.

Table 3. Metabolic rate ranking of five loblolly pine seed orchard clones (sample date = 3/6/90).

Clone Height PL	Metabolic Rate, .tW/mg
81	**10.6**
79	10.3
77	8.8
15	7.2
30	7.1

CONCLUSIONS

The results presented here are the first reported measurements of metabolic rates of loblolly pine. The value of metabolic rate as a selection tool holds considerable promise, especially due to the apparent relationship of metabolic rate to shoot growth cycles. In loblolly pine, shoot cycles, environmental stresses, and the differentiation of reproductive structures must be taken into consideration before genetic comparisons are made. Metabolic rate measurements may lead to a better understanding of the dynamics of shoot formation itself, perhaps by providing information on the intensity and persistence of metabolic activity during stem unit initiation. In addition, the sensitivity of metabolic rate to environmental stress that was strongly implicated here, and has been demonstrated elsewhere (Criddle et al., 1988), may prove useful in identifying trees that can maintain high metabolic rate under adverse conditions.

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