Temperature Effects on Oxidative Metabolism of Dormant Sugar Pine Seeds

J. BRAD MURPHY AND THOMAS L. NOLAND
Department of Horticulture and Forestry, University of Arkansas, Fayetteville, Arkansas 72701

ABSTRACT

When dormant sugar pine (Pinus lambertiana L.) seeds were imbibed at 5°C, they showed a rapid increase in O2 uptake, ATP level, and moisture content during the first 4 days. This was followed by a plateau phase until 60 days, after which a second significant increase in all three features occurred as dormancy was broken. During the plateau phase, conventional CN-sensitive respiration accounted for 74 to 79% of the total O2 uptake. When dormant sugar pine seeds were imbibed and maintained at 25°C, a different pattern occurred. Water uptake was much more rapid during the first 4 days and no second increase occurred after 60 days because the seeds did not break dormancy. There was an initial burst of O2 uptake and ATP formation, but these both declined abruptly after 24 to 48 hours. Levels about half of seeds at 5°C were maintained through the rest of a 90-day period. CN-sensitive respiration declined during imbibition at 25°C, and accounted for only 55 to 61% of the total O2 uptake. The inability of dormant sugar pine seeds to germinate at temperatures above about 17°C may therefore result from initial temperature effects on membrane properties, leading to reduced O2 uptake, reduced cytochrome oxidase electron transport activity, and lowered ATP levels.

There is an extreme paucity of information about basic metabolic changes occurring during stratification of dormant pine seeds. We have determined previously that changes in inhibitor content during stratification of sugar pine seeds could not be causally related to loss of dormancy (12, 13), which supports current concepts that membrane properties or regulatory metabolism may also be involved in the control of seed dormancy and germination (7, 11).

We recently observed that the temperature during imbibition of sugar pine embryos had a significant effect on rates of water influx and solute efflux. Temperatures above 17°C, at which sugar pine seeds do not normally germinate, caused abnormally high rates of water uptake and solute leakage to occur, possibly due to temperature effects on membrane properties (14). This appears similar in nature to the injurious effect of elevated temperatures and humidity in accelerated aging treatments (1, 15) and the deleterious effect of cold temperatures on chilling-sensitive species (8, 10, 17). In these cases, apparent membrane damage appears to be an initial step in a series of events leading to abnormal metabolism. Electron transport and phosphorylation are decreased, respiration and the ATP levels are reduced, numerous metabolic pathways become impaired, and toxic metabolites may accumulate, all leading to a reduction of growth or death (1, 10). The purpose of this investigation was to determine whether a similar series of metabolic dysfunctions occurs at the moderate temperatures at which the apparent effect on membrane properties was observed in sugar pine embryos.

MATERIALS AND METHODS

Seeds of sugar pine (Pinus lambertiana L.) were obtained from Schumacher (Sandwich, MA). Intact seeds were imbibed in 5 mM phosphate buffer (pH 7.2) for 24 h at either 5 or 25°C. Blotted dry, and then treated with 0.1% (w/w) Arasan 50-Red (DuPont). Seeds were then placed in plastic bags with moist paper towels and maintained at either 5 or 25°C. At 1, 2, 4, 7, 14, 30, 60, and 90 d, embryos were excised from the seeds for determination of moisture content, respiration rates and pathways, and ATP levels. The possible effect of an initial period of total immersion was tested by a parallel experiment in which dry seeds were simply placed on moistened germination blotters (Anchor Paper Co., St. Paul, MN) at either 5 or 25°C.

Moisture content was determined gravimetrically and expressed as percent fresh weight. Respiration rates were measured polarographically with a Clark oxygen electrode (Yellow Springs Instrument Co.). O2 uptake of five excised embryos was measured for 15 min in 6 ml 5 mM phosphate buffer (pH 7.2) containing 25 μg/ml streptomycin sulfate, in a thermostatted circulation chamber at 25°C. Evidence of activity of particular respiratory pathways was obtained by the use of inhibitors: 1 mM KCN, and 1 mM SHAM2 and PG combined (16, 18, 19). Excised embryos were soaked for 2 h at 25°C in buffer (control) or fresh inhibitor solutions before measurement of O2 uptake. ATP was extracted with boiling water (4), and quantified by the bis luminescent luciferin-luciferase assay (21). Luminescence was measured on a Lumi-Tran ATP photometer (New Brunswick Science Co.). Respiratory rates and ATP levels are expressed on an ODW basis. Data presented are averages of duplicate samples from two separate experiments.

RESULTS

Initially, there were measurable differences in water uptake between seeds imbibed at 5 or 25°C (Fig. 1). After 24 h, the 25°C embryos contained 26% more water than the 5°C embryos. By day 14, the 5°C embryos had reached approximately the same level of hydration as the 25°C embryos, and no substantial differences reappeared until after 60 d. By this time, the 5°C seeds had broken dormancy and begun a second phase of water uptake. A similar initial pattern occurred with seeds which were not initially immersed, but placed on moistened germination blotters (data not shown).

1 Supported by the McIntire-Stennis Cooperative Forestry Research Program. Published by permission of the Director of the Arkansas Agriculture Experiment Station.

2 Abbreviations: SHAM, salicylhydroxamic acid; ODW, oven-dry weight; PG, propyl gallate.

Received for publication March 3, 1982 and in revised form June 20, 1982
Temperature during imbibition had a significant effect on rates of O₂ uptake by the embryos (Fig. 2). At 24 h, the 25°C embryos were respiring at a rate 26% greater than the 5°C embryos. After 48 h, the 25°C embryos began a rapid decline in O₂ uptake which leveled off by day 7 to a relatively slow linear decline through the rest of the treatment period. At 5°C, the embryo O₂ uptake rate increased rapidly until day 4, then leveled off until after 60 d, after which a dramatic increase in O₂ uptake occurred, associated with loss of dormancy of the seeds. A similar pattern occurred with nonimminated seeds (data not shown).

The pattern of CN-sensitive O₂ uptake paralleled that of total O₂ uptake (Fig. 2). The proportion of the total O₂ uptake that was due to the conventional Cyt oxidase electron transport system (CN-sensitive) or alternative pathways (CN-insensitive, SHAM- and PG-sensitive) was significantly affected by temperature (Fig. 3). On day 1, the proportions of CN-sensitive and SHAM- and PG-sensitive respiration were identical at the two temperatures. Thereafter, at 5°C, CN-sensitive respiration accounted, on the average, for 79% of the total O₂ uptake, compared to only 62% at 25°C. The average proportion of the total O₂ uptake due to an alternative SHAM- and PG-sensitive respiration was only 8% at 5°C, compared to 26% at 25°C. At both temperatures, there was a similar residual O₂ uptake (about 12% of the total) which was apparently insensitive to all three inhibitors.

Upon initial imbibition, the level of ATP in the embryos increased significantly at both temperatures (Fig. 4). At 24 h, however, the ATP level at 25°C was 32% greater than at 5°C. This was followed by an abrupt decline in the ATP level at 25°C, while at 5°C the level of ATP remained relatively constant until after 60 d, when a second increase in ATP began. Between 60 and 90 d at 5°C, the level of ATP doubled as the seeds came out of dormancy. During the majority of the treatment period, the level of ATP at 5°C was at least twice that at 25°C, reflecting the similar pattern in O₂ uptake.

**DISCUSSION**

Seed dormancy is defined as the inability of viable seeds to germinate, even when placed in favorable conditions. Stratifica-

**Fig. 1.** Cumulative water uptake of sugar pine embryos excised from whole seeds which were immersed 24 h and maintained at either 5°C (U) or 25°C (●).

**Fig. 2.** Respiratory activity at 25°C of sugar pine embryos excised from whole seeds which were immersed for 24 h and maintained at either 5 or 25°C: total O₂ uptake of 5 (○) or 25°C (●) seeds, CN-sensitive (1 mM KCN) O₂ uptake of 5 (○) or 25°C (●) seeds. After 90 d at 25°C, there were not enough sound seeds to make a determination.

**Fig. 3.** Per cent contribution of CN-sensitive (○, ○) and SHAM- and PG-sensitive (●, □) respiration to total O₂ uptake of sugar pine embryos excised from whole seeds which had been immersed for 24 h and maintained at either 5°C (○, □) or 25°C (●, ●). After 90 d at 25°C, there were not enough sound seeds to make a determination.
The present study did not allow the determination of the exact pathways of CN-insensitive $O_2$ uptake. As there was a significant increase both in the absolute levels and the proportion of total $O_2$ uptake due to CN-insensitive pathways at $25^\circ C$, the pathway(s) may be of consequence to the metabolic differences observed. The possible activity of lipoxigenase would be of particular interest, as a potential contributor of oxy free radicals (9), which could further disrupt membrane properties and mitochondrial activities. To our knowledge, this is the first instance in which fairly moderate temperatures have been shown to have such a rapid deleterious effect on plant metabolism. Dormant sugar maple seeds imbibed at $20^\circ C$ showed a decline in respiration and ATP levels, but only after a prolonged period of 10 to 22 d (20). While our results do not explain what processes occur during stratification which allow sugar pine seeds to move out of a dormant condition into a growing one, they provide a possible explanation as to why dormant sugar pine seeds are unable to germinate at warmer temperatures.

**LITERATURE CITED**

5. HENDRICKS SB, RB TAYLORSON 1976 Variation in germination and amino acid leakage of seeds with temperature related to membrane phase change. Plant Physiol 58: 7-11
20. SIMMONDS JA, EB DUMBERTOFF 1974 High energy charge as a requirement for axis elongation in response to gibberellic acid and kinetin during stratification of Acer saccharum seeds. Plant Physiol 53: 91-95