

SEASONAL VARIATION IN MITOTIC INDEX IN THE STEM APEX
OF LOBLOLLY PINE SEEDLINGS

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Abstract.--The mitotic index of the stem apex of loblolly pine seedlings was studied over a one year period from age 4 months to age 16 months. Mitotic index ranged from approximately 3% midwinter to over 17% midsummer. Comparative studies of 3 loblolly pine open pollinated families showed significant differences in mitotic index at several points in the late September to early March time period. These studies are discussed in terms of bud dormancy status of loblolly pine.

Dormancy status of the terminal bud is an important factor of seedling quality. The terminal bud is the site of chilling perception (Romberger 1963) and studies indicate that many north temperate conifers either have an obligate (i.e. chilling is mandatory for budburst) chilling requirement for budburst (Douglas-fir, Wommack 1964) or require chilling for the normal rate of budburst in the spring (Garber 1978, Greenwood 1978-81). Several studies have shown that chilling seedlings alters their dormancy status and root regeneration potential (Stone et al 1962, Lavender 1964, Ritchie 1981). Both root growth potential and seedling survival after outplanting have been observed to peak midwinter; these midwinter peaks are theorized to be related to seedling dormancy status. Determination of seedling dormancy status has in most studies (such as those cited above by Stone, Lavender and Ritchie) involved placing seedlings in a spring-like environment (i.e. warm, wet, long-day) and noting the number of days to terminal budburst (DBB).

Dormancy status is affected by changes in environmental factors such as exposure to chilling. Thus, since it takes several weeks to determine the time to budburst the dormancy status of the crop may have changed, preventing direct use of the information for crop management. The most common method of predicting dormancy status of nursery crops is from the accumulation of chilling hours. There are no usable methods which allow the rapid prediction of current dormancy status from a direct measurement of the crop seedlings.

Dormancy in forest trees can be described in terms of such shoot elongation phenomena as DBB or cell divisions in the terminal buds or lateral cambia. In some forest trees, cell division in vegetative buds stops for a period during midwinter (Owens and Molder 1973, Cottignies 1979). Carlson et al. (1980) compared shoot-elongation dormancy with dormancy described as lack of cell division in the terminal buds of Douglas-fir seedlings. The beginning of the period of deep dormancy (where chilling was required prior to resumption of shoot elongation in a warm, moist, long-day environment) coincided with cessation of cell division in the terminal bud.

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The percentage of cells in the terminal bud undergoing division (i.e. the mitotic index) can be estimated within a few hours. Mitotic index as an estimate of dormancy status could therefore be used directly to manage the seedling crop. Interactive measurement of dormancy and crop management could be a valuable tool in improving crop quality.

The purpose of this study was to provide baseline information concerning the annual cycle of cell division in the terminal buds of one loblolly pine seed source, and to provide an estimate of variation in mitotic index between 3 open-pollinated families of loblolly pine.

METHODS

Loblolly pine seed of a northern Louisiana source was sown in early April 1981. Seedlings were cultured as bareroot nursery stock using standard operational procedures at Weyerhaeuser Co. Fort Towson Nursery in southeastern Oklahoma. Biweekly bud sampling was begun in August 1981 and continued for one year, 16 buds were sampled at each date. Results from a preliminary study showed that the most variable periods of mitotic index were during daylight therefore all bud sampling was done in predawn hours. Air temperature (20cm above the ground) was recorded at the time of sampling.

A second study was done in the fall and winter of 1982-83. Seedlings of 3 open-pollinated families of loblolly pine were sampled from a multifamily comparison test plot. In these test plots adjacent double rows (8 double rows/nursery bed) were sown with seed of different families. Replicate plots were 32m long and 1.2 m (one bed) wide. Terminal buds of each family were sampled from seven replicate plots sampled biweekly from September 15, 1982 - March 1, 1983. Bud samples were pooled for each family.

Buds were sampled into McClintock's solution and unless otherwise noted processing followed the methods of Carlson et al. 1980. The entire bud and distal few millimeters of the shoot were fixed in the field. Later, the buds were dissected and the apical meristem removed for preparation of the squash.

Immediately after staining, cells were counted using a microscope equipped with a 10 x ocular, a 40 x objective, and an ocular counting grid. all of the stages of karyokinesis were counted as dividing except interphase. To determine the onset of bud dormancy more precisely, the least dormant area of the squash was counted. Mitotic index is defined as the number of dividing cells divided by the total number of cells.

RESULTS

Annual cycle.--Mitotic index in terminal buds of loblolly pine seedlings varied significantly with sampling date ($P = 0.0001$). A distinct annual cycle of mitotic index was apparent (Figure 1). Mitotic index varied from a maximum of 18% in late June to a minimum of 3% in early February. Chilling hour accumulation (Figure 2) was inversely related with mitotic index from October through January. Air temperature at time of sampling (Figure 3) was not related to the annual mitotic index cycle; however, the general air temperature (monthly mean) pattern (Figure 4) does resemble the mitotic index pattern. The reason mitotic index follows long term temperature trends could be that the cell division cycle lengthens considerably at common winter temperatures (Figure 5).

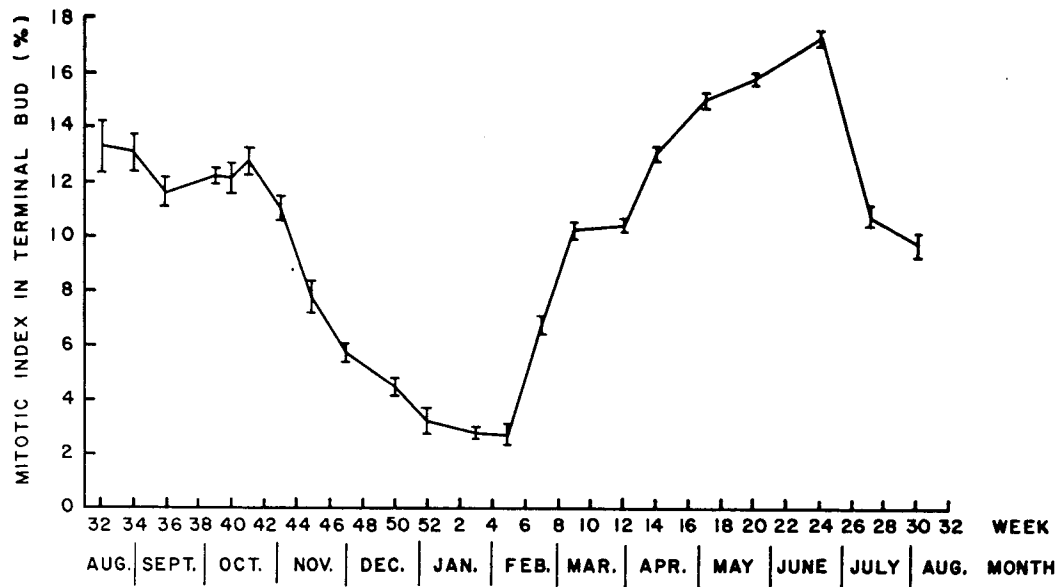


Figure 1. Annual course of mitotic index in loblolly pine stem apices. Means are shown \pm one standard error of the mean.

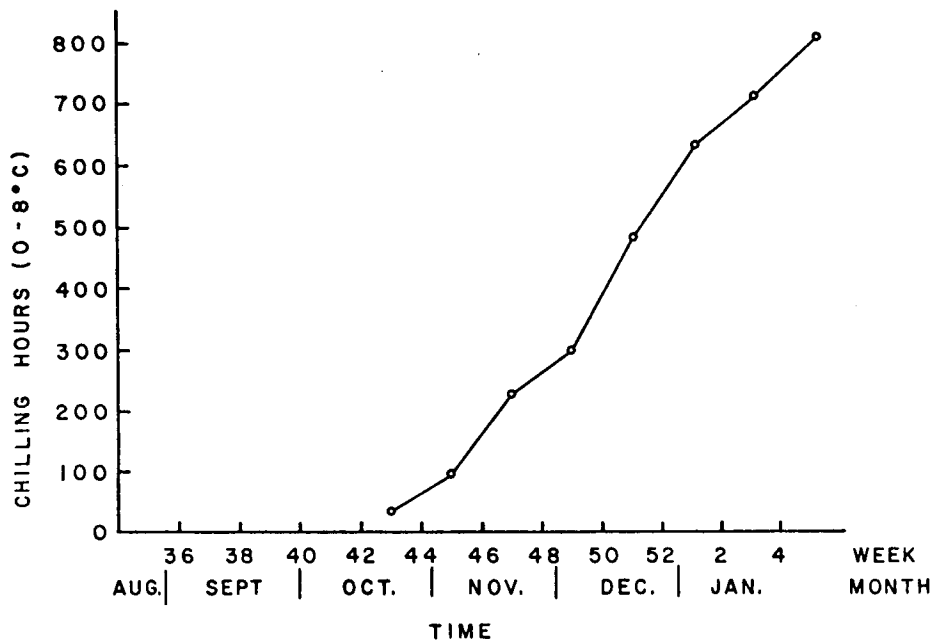


Figure 2. Accumulation of chilling hours from October 15, 1981 to February 1982.

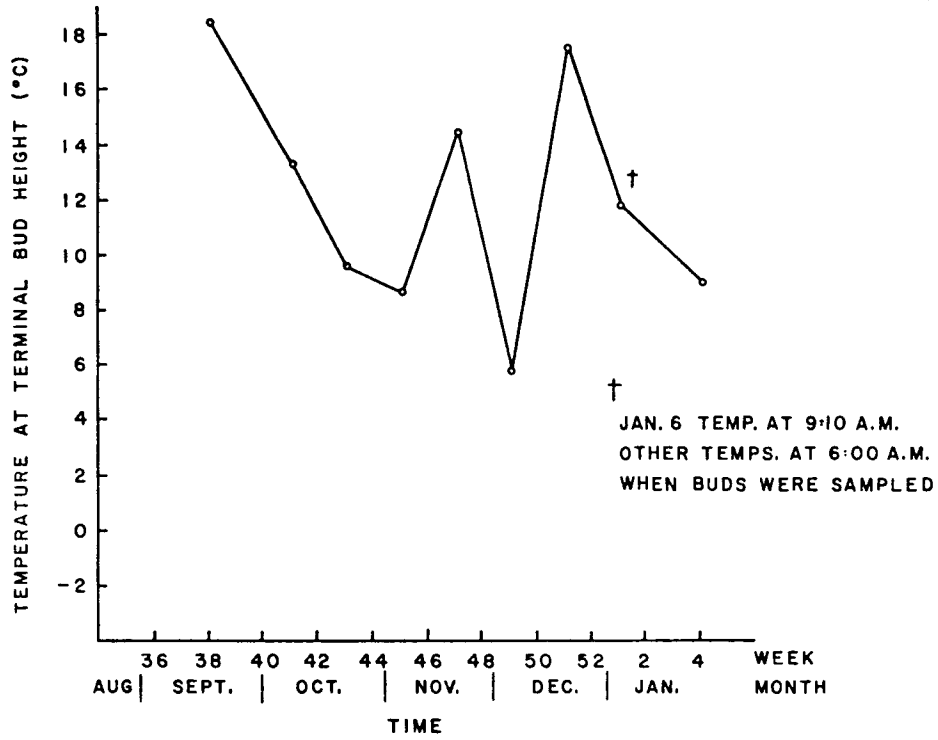


Figure 3. Temperature 20 cm above the soil at time of bud sampling from Sept. 1981 through January 1982.

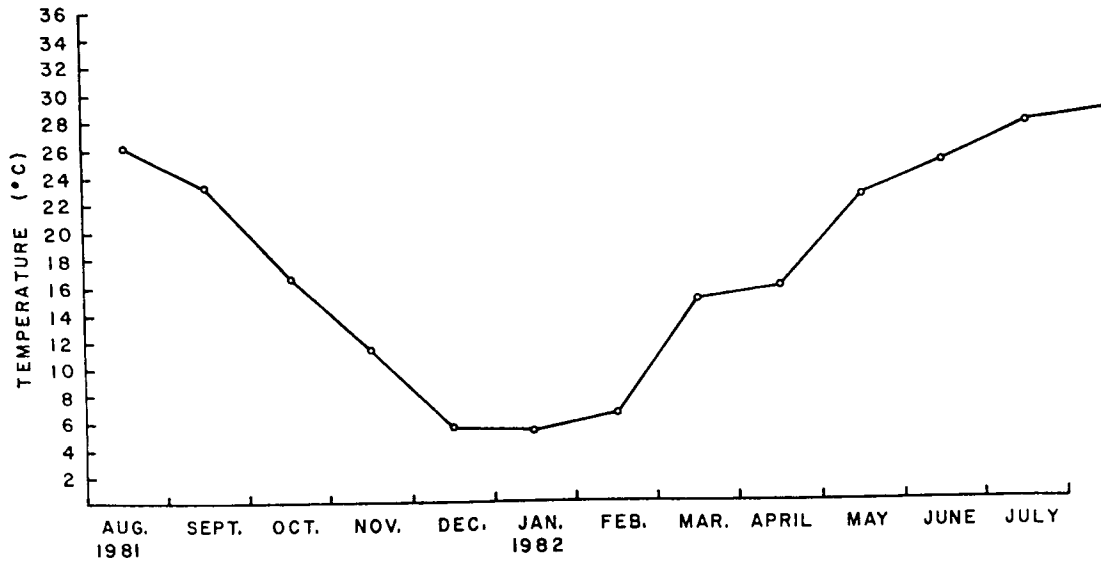


Figure 4. Annual course of air temperature in southeastern Oklahoma.

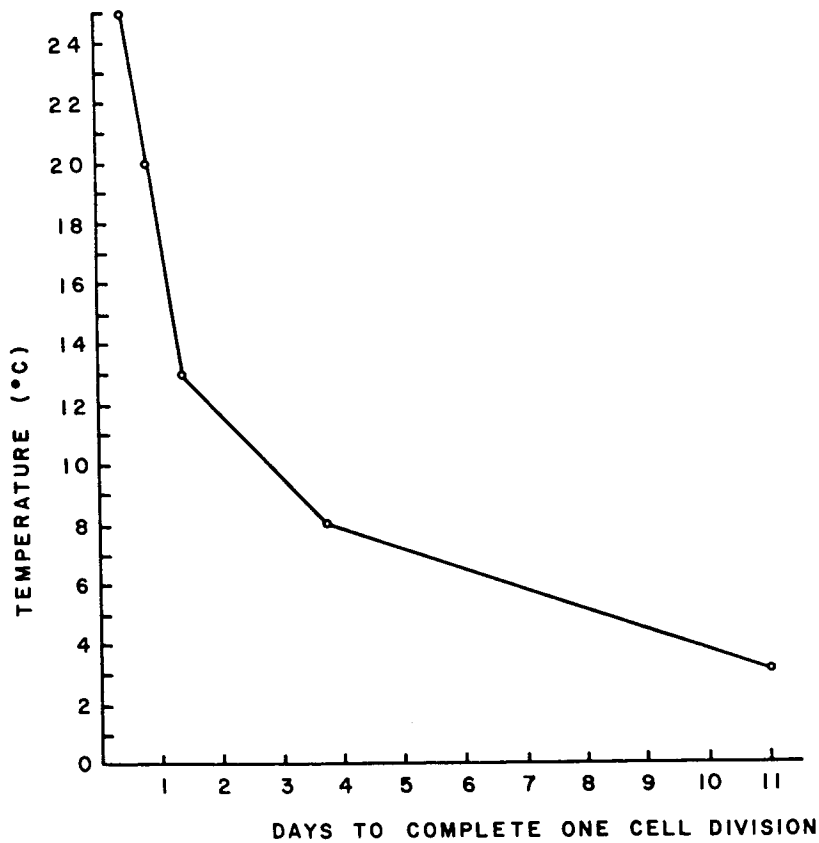


Figure 5. The relationship between temperature and the length of the cell cycle in root tissues of Vicia faba. Drawn from data presented by Murin (1981).

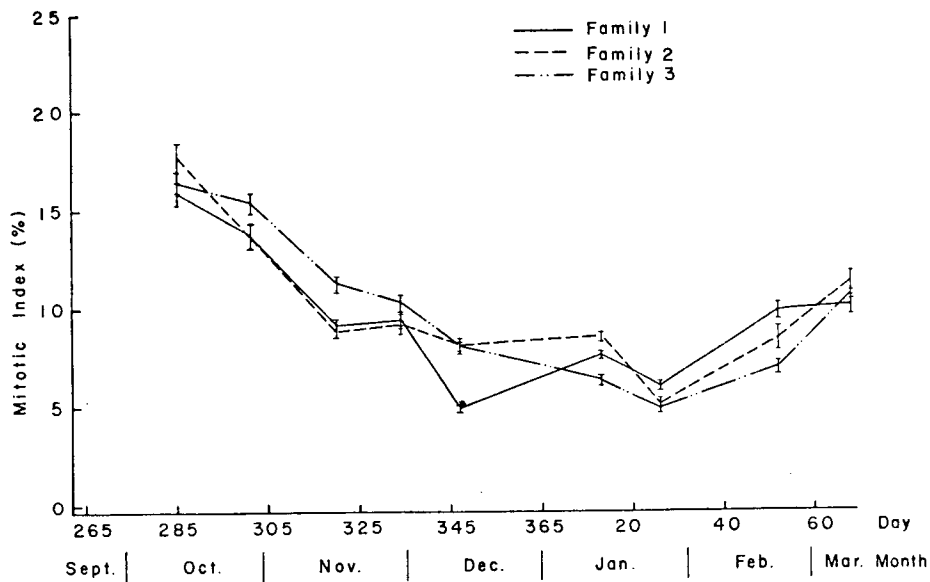


Figure 6. Comparative mitotic indices in the stem apices of seedlings from three open pollinated loblolly pine families. Means are shown \pm one standard error of the mean.

Comparison of families.--The three families tested in 1982-83 showed differences in pattern of fall-winter mitotic index (Figure 6). Family 3 tended to have higher MI than the other families thru the fall, had minimal MI in late January then a trend lower than other families into March. In contrast, family 1 trended lower than other families thru the fall with a minimum in early December, followed by a comparatively high trend in January and February. Family 2 followed a similar pattern to 1 until the end of November, then remained relatively high until mid January. Minimum MI for family 2 occurred in late January followed by an increasing rate intermediate to the other families until mid February. Family differences were statistically significant ($\alpha = 0.05$) on Nov. 16, Dec. 13, Jan. 13, Jan. 26, and Feb. 21.

DISCUSSION

Determination of dormancy status of seedlings is very important to management of nursery crops. Managers must be able to determine the dormancy status of seedlings in order to properly schedule cultural practices in the nursery, and lifting and storage activities. The most common method of determining dormancy status is noting the number of days required for terminal budbreak in a warm, wet, long day environment. This procedure requires several weeks, therefore allowing the crop in the field to change status by accumulation of chilling, before test results are available for use in management. Dormancy status is predicted year to year by chilling hour accumulation. Determination of mitotic index requires only a few hours, and therefore could be used to measure current crop status. It could be useful in comparing the dormancy status of different portions of a nursery crop managed under different cultural regimes. For example, Carlson *et al.* (1980) showed that application of nitrogen increases the mitotic index of Douglas-fir seedlings.

Loblolly pine seedlings showed marked annual fluctuation in mitotic index in the stem apex but continued a low level of division even in mid winter. This is especially notable since these studies included only the stem apex whereas Carlson *et al.* (1980) studied the entire terminal bud of Douglas-fir and found that cell division dormancy occurred in early December. In Douglas-fir cells in the apex cease division earlier than those of the more distal leaf primordia (Owens and Molder 1973).

There are several important points in the mitotic index curve; (1) division falls off following the longest photoperiod, (2) another major point of rapid reduction coincides with mid-October when Garber's (1978) work indicated that the seedling begins chilling hour accumulation, (3) mitotic index was inversely related to the accumulation of chilling hours, perhaps reflecting the average effect of reduced temperature on cell division cycle time (Grif 1967, Murin 1981), (4) the period of minimum mitotic index extends from early January through mid-February, the period of most successful transplanting, (5) a rapid rise in mitotic index in late February and early March coincides with observations of reduction in seedling quality, and (6) the mid-March plateau coincides with both budburst and the equinox.

The cell cycle progresses through 4 basic stages, G_1 (pre DNA synthesis), S (the time of doubling of DNA) G_2 (a post-DNA synthesis) but premitotic period and finally M, the period of mitosis (Howard and Pelc 1953). G_1 is usually the longest phase. Cellular susceptibility to damage or disruptive processes is considered lowest in G_1 phase.

Factors regulating changes in mitotic index over the season are not well understood. Reduction of temperature lengthens the cell cycle (Brown 1951, Grif 1967). Murin (1981) presented data for Vicia faba roots that shows a nonlinear temperature relationship (figure 5). Seasonal monthly temperature means for SW Oklahoma are shown in figure 5. While it is tempting to note a cause-effect relationship, Murin noted that all stages of the cell cycle are reduced proportionately when the temperature is reduced. If this is true for loblolly pine then reduction in temperature would not change mitotic index since all parts of cell cycle would still proceed proportionately, just at a lower rate.

Another explanation of seasonal changes in mitotic index is that a majority of cells stop cycling during midwinter. Owens and Molder (1973) found that Douglas-fir vegetative bud cells stop cycling predominantly in G_1 although there was a small population in G_2 . In Fraxinus excelsior the shoot apex of the terminal bud arrests in G_1 during winter dormancy (Cottignies 1979). Similarly, in seeds of both Pinus pinea (Brunori and d'Amato 1967) and Pinus silvestris (Thomas 1975) dormant meristems are in G_1 phase.

Further studies will be necessary to determine whether loblolly pine exhibits midwinter cell division arrest in G_1 , therefore having properties of dormancy in common with Pseudotsuga and Fraxinus. The 2.8% division midwinter could represent a specific subpopulation of cells that differ in cell cycle properties. A second and perhaps more likely possibility is that under the influence of temperature and/or photoperiod the cell cycle time lengthens in loblolly pine without a specific G_1 arrest point. This would be a departure from the pattern observed in the colder climate species mentioned above but might explain some loblolly pine characteristics such as the tendency toward midwinter budscale slippage in warm periods. Since midwinter cell division occurs and budbreak is preceded by a rise in mitotic index, such midwinter cell division could explain a higher rate of response to warm midwinter temperatures, and perhaps to a nonobligate chilling requirement.

Family variation in mitotic index was significant at several points in the fall-winter period. More research is necessary to determine whether family variation in mitotic index is indicative of differences in physiological quality.

Specifically, more research is also needed to determine the relationship between cell division in the terminal bud, chilling perception and dormancy status with regard to root growth potential. If these relationships are strong, then determination of mitotic index could be a rapid means of assessing crop status prior to making a decision to lift and/or store seedlings.

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