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The objectives of this investigation were to measure the seasonal and diurnal variations of starch as well as to relate these variations to growth of the trees. This study also microscopically examined the needles to determine if starch exists in granular form in Douglas-fir. The objectives were designed to gain some insight into the patterns of energy storage in young growth Douglas-fir, and its role in seasonal growth.

Diurnal starch variation followed a cycle of synthesis and depletion with three peaks in concentration; one around noon, another in early evening, and the third one during the early morning hours. Seasonal starch concentrations peak in the spring before bud burst. Following bud burst, starch concentration decreased. Higher levels of starch were observed in the upper crown than the lower crown level. Starch was shown to exist in the form of granules in the chloroplasts of cells in the needles of Douglas-fir. In the early evening lipid droplets were observed within the chloroplasts, nowever by early morning no visible sign of these droplets was to be seen.

Starch Content and Crown Growth in Two Douglas-Fir Stands

by

Kran James Kilpatrick

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STARCH CONTENT AND CROWN GROWTH IN TWO DOUGLAS FIR STANDS

INTRODUCTION

Plant vigor is believed to be directly correlated to energy reserves (Kozlowski, 1964), with high reserves indicating a healthy tree, resistant to stresses placed on it (Wargo, Parker and Houston, 1972). Since plant growth is partially dependant on energy reserves, seasonal and even diurnal, or daily, variation in starch levels may be useful for estimating growth potential. Such information about energy storage may be used, for example, to help assure the survival of seedlings after lifting or to determine the proper time to thin a forest to allow for maximum growth of the remaining trees. Girdling of small branches prior to their removal for rooting, allows reserve energy accumulation which aids in root establishment.

Energy reserves in plants are composed of carbohydrates, starch, fats, oils, and other similar compounds. One of the most important classes of energy reserves is carbohydrates because they are direct photosynthetic products and hence materials from which proteins and fats are synthesized (Little, 1970a).

Carbohydrates move through the tree in the form of sucrose or sucrose-containing oligosaccharides (Trip et. al., 1965), with starch apparently serving as the main storage form of carbohydrates in conifers (Kimura, 1969). Starches are expected to be good indicators of carbo-

hydrate changes in the plant, since sugar-starch transformations act to maintain the sugar pool not the starch level, at a relatively constant level (Kozlowski and Keller, 1966).

The purpose of this study was to sample and document starch levels in young Douglas-fir trees in stands, since the only previous research on starch in Douglas-fir had been done on seedlings (Kruger and Trappe, 1967). In this study, I examined the diurnal and seasonal variation in starch levels in the bark and foliage of the Douglas-fir.

Specifically I:

Measured the diurnal fluctuation of starch in the bark and foliage of Douglas-fir.

Measured the seasonal variation of starch in Douglas-fir bark and foliage in two stands.

Determined that starch is present in the form of granules in the foliage of Douglas-fir.

Measured lateral growth of branches in two Douglas-fir stands, one at 500 meters and the other at 1128 meters elevation.

Analyzed the data collected on starch and growth to determine if they are related.

These objectives were designed to gain some insight into the patterns of energy storage in young Douglas-fir, and its role in seasonal growth. Understanding these patterns of energy storage in a young stand of Douglas fir could aid in the management of these stands. This study provides a baseline of starch content information on healthy trees.

LITERATURE REVIEW

I reviewed published literature on the composition and appearance of starch; its role in the storage of energy; the seasonal fluctuations of starch and photosynthesis, and the diurnal variations of photosynthesis. Most studies on starch have been done on food crops such as grains and a review of this literature has not been included since it is voluminous and not relevant to this study.

There are probably more carbohydrates in the biosphere than all other organic matter combined, largely because of the abundance of the two polymers of D-glucose, cellulose and starch (Lehninger, 1970). It is well established that starch is composed of two chemically and physically distinguishable polysaccharide fractions, α-amylose and amylopectin. The ratio of α -amylose and amylopectin in plant starch is determined genetically (Banks and Greenwood, 1975).

α-Amylose consists of long unbranched chains in which all the D-glucose units are bound in α (1$\rightarrow$4) linkages with a helical conformation. The chains vary in molecular weight from a few thousand to 500,000. Amylopectin, which lacks the long helical glucose chains, is highly branched. The branches occur at every twelfth glucose residue and are about twelve glucose residues long. The backbone glycosidic linkage is α (1$\rightarrow$4), but the branch points are α (1$\rightarrow$6) linkages. Whelan proposed that the fine structure of the amylopectin molecule is

more complicated than previously accepted structures (Whelan, 1971, Lee and Whelan, 1971). Amylopectin has molecular weights ranging up to 1,000,000 (Akazawa, 1976).

Starch deposited in plant cells appears as granular particles (Hilliard, 1970; Haapala, 1968; Akazawa, 1976; Lehninger, 1970). Different plant species have their own specific starch granules, varying in such characteristics as size, shape, and structure of the shells, although starch molecules are identical in their chemical architecture. Through microscopic examination it is often possible to identify the source of starch (Reichert, 1913) and to study the dynamic features of starch granule formation in plant cells as suggested by the layer arrangement or shell structure (Buttrose, 1960, 1962; Street and Cockburn, 1972).

Starch in plants is considered to be the chief form of stored energy (Lehninger, 1970; Akazawa et al., 1964; Hess, 1975), and is produced by chloroplasts in cells in the leaves of plants. The photosynthetically-produced starch accumulated in chloroplasts, often called assimilation starch, is a transitory reserve carbohydrate (Akazawa, 1976).

In trees, starch is the most abundant reserve polysaccharide (Kramer and Kozlowski, 1960; Gibbs, 1940; Preston and Phillips, 1911; Siminovitch et al., 1953). During a time of stress on the tree, i.e. defoliation by insects, starch levels decrease (Parker and Patton, 1975; Wargo et al., 1972; Webb and Krchesy, 1976; Hepting, 1945).

Seasonal changes in starch and sugar concentrations in various tissure and organs have been documented in a wide variety of conifers

(Krueger, 1967; Kozlowski and Keller, 1966; Parker, 1959; Whetter and Taper, 1963; Krueger and Trappe, 1967; Little, 1970a, 1970b). In general, sugar is high in the winter and low in the summer, whereas starch is high in the spring and low throughout the rest of the year. Starch concentration increases dramatically during the one to two month period immediately prior to bud burst (Krueger and Trappe, 1967; Kimura, 1969; Little, 1970b). After bud burst, vigorous shoot growth begins and starch content decreases rapidly. The rapid springtime increase in starch may arise from either current photosynthate or conversion of photosynthates produced during the previous year and stored as either sugars or fats.

Starch is one of the first products of photosynthesis, and therefore variations in rates of photosynthesis should result in similar variations in starch content. Several studies have been conducted on seasonal fluctuations in photosynthesis of trees (Polster, 1950; Negisi and Satoo, 1961; McGregor and Kramer, 1963). Photosynthesis in Pinus strobus seedlings was low in May and June, and rose steadily to a maximum for the year in September. Thereafter rates decreased, but substantial rates were recorded in October and November (Nelson, 1964). Using Pinus cembra, at timberline, Tranquillini (1959) showed that high rates of photosynthesis were occuring in late May. In the autumn photosynthesis ceased long before snow covered the plants.

Until recently the concept of winter photosynthesis in evergreens has been subject to debate. Freeland (1944) observed in several Pinus species that some photosynthetic activity occurs in winter and Hepting (1945) found that carbohydrate reserves in Pinus echinata increased

significantly in winter. Others have shown this small but detectable persistence of photosynthesis to occur throughout most of the winter. Helms (1965) stated that net photosynthesis occurs during the winter and contributes significantly to stored energy reserves. This net accumulation, prior to the flush of spring growth, may be of considerable importance to the overall energy economy of Douglas-fir (Kramer, 1957; Parker, 1961).

During certain seasons, growth of conifers is sustained by current photosynthate, but stored energy is also important for spring shoot elongation (Allen, 1964; Kozlowski and Winget, 1964). Concentration changes, revealed by staining techniques, have been seen as evidence that starch converts to fats in conifers as temperatures decrease in autumn (Hilliard, 1970). Marvin et al. (1971) demonstrated in Acer that starch content decreased and sugars increased during the cold months, with the reverse occurring during the warm months. The maximum starch concentration in woody plants was in the spring around the time of bud burst (Eifert, 1963; Krueger and Trappe, 1967; Little, 1970a, 1970b; Siminovitch et al., 1953). This starch reserve that accumulates prior to bud burst provides only an insignificant part of the carbohydrates required for current shoot growth. Current shoot growth in balsam-fir seedlings was not related to starch level at budbreak (Little, 1974). Reserves of carbohydrate accumulated the previous year after shoot elongation has ceased, apparently are important to current shoot growth in conifers (Olofinboba and Kozlowski, 1973).

There do not appear to be any studies on the diurnal fluctuations of starch in conifers, but there is much literature on diurnal fluctuations in photosynthesis. Photosynthetic rates vary greatly during the day, exhibiting patterns similar to those for net assimilation. It is believed that these patterns are either an inherent function of the photosynthetic mechanism or a more complex change in the internal status of the tree (Polster, 1950; Miller, 1959; and Helms, 1965). The maximum rate of photosynthesis is achieved before noon and is often followed by a midday depression, recovery in the late afternoon, and a final decline late in the day (Polster, 1950; Tranquillini, 1954; Kramer and Kozlowski, 1960; Helms, 1965; Heiniche and Childers, 1937; Parker, 1953; Kurssanow, 1933, 1934; Brix, 1962; Kozlowski, 1964; Ah-Sing Chia-Looi and Cumming, 1972; Pallas et. al., 1974).

Diurnal variations of starch within a plant may be a result of many factors. Internal factors such as water stress (Brix, 1962; Kozlowski, 1964), stomatal closure (Nutman, 1937), excessive transpiration (Tranquillini, 1954), and the accumulation of end products (Kurssanow, 1933, 1934) appear to influence diurnal rates of photosynthesis (Waugh, 1939). External factors such as light (Haapala, 1969; Bormann, 1953; Kramer, 1958; Eagles, 1967), CO_2 concentration of the air (Chapman, Gleason and Loomis, 1954; Huber, 1958) and temperatures (Hilliard, 1970) also exert a controlling influence. Diurnal variations in starch may be the result of any one of these factors, but it is more likely the result of a combination of several factors.

Many other phenomenon in plants have been shown to have diurnal variations. Durzan (1967) reported diurnal changes of amino acids, amides, protein, and chlorophyll in the needles of white spruce Picea glauca. Parsons and Kramer (1973) reported a diurnal cycling in root permeability to water. Diurnal variations which occur in different systems of a plant are important for measuring short term responses of plants to their environment.

No studies have been recorded on a lag in time between starch appearing in the needles and then being translocated into the bark in Douglas-fir. One study (Mason and Maskell, 1928) did report a lag in the diurnal fluctuation of sugars between the foliage and bark. Variation of starch levels in different parts of the canopy has not been reported but will be examined in the present study.

METHODS

The objectives of the study were to determine if starch is present in chloroplast granules in the needles, to measure diurnal and seasonal variations of the starch content in Douglas-fir bark and needles, and to correlate starch estimates with current growth. The seasonal study was done in two stands of young Douglas-fir.

The diurnal starch measurements were conducted on an open grown tree with branches extending to the ground, located next to the Forest Research Laboratory at the Oregon State University campus in Corvallis, Oregon. The seasonal measurements were made on two stands at different elevations on Mary's Peak, 15 miles southwest of Corvallis, Oregon.

Field methods

The diurnal study was begun at 6:30 P.M. on May 21 and continued for twenty-four hours. On that day the sun rose at 4:39 A.M. and set at 7:41 P.M. for a day length of fifteen hours. During the twenty-four hour period, there was no precipitation or cloud cover. The tree was in an active growth phase since most of the buds had just opened or were swelling. Samples were taken every two hours starting at 6:30 P.M., 8:30 P.M., etc., from branches located on the southeast side of the tree at the fourth, eighth, and twelfth whorl down from the top which were used to represent the upper, mid, and lower crown levels respectively. The samples, consisting of short twigs that measured less than 15

centimeters in length, were frozen immediately on dry ice. Additional samples were taken at this time for later electron microscopy observation to determine the presence or absence of starch grains.

The samples for the seasonal portion of the study were taken at approximately two week intervals beginning on March 15, with the sampling dates listed in Appendix A. On each plot ten dominant trees were selected for similar height and DBH and marked with colored flagging. Five of these trees were picked randomly for sampling. The fourth, eigth, and twelfth whorl from the top were marked and used to represent the upper, mid and lower crown levels, respectively. The twelfth whorl was near the bottom of the crown. The branch closest to the southeast side of the tree was used for sampling. On the upper plot the mean height of the trees was 9.4 meters and the mean age was 26.4 years; the lower plot mean tree height was 15 meters and the mean age is 25.8 years. Many of the trees on the upper plot had multiple leaders; the lower plot trees were rapidly growing and most had a well developed leader. Samples consisting of twigs approximately 15 centimeters were collected at random locations on each branch on each sampling date. The samples were sniped off the branches with pruning shears, placed in plastic bags, labeled and put on dry ice immediately for transportation to the laboratory. Sampling began one hour before sunrise and was halted one half hour after sunrise when photosynthetic activity begins to increase. Lateral growth was measured in centimeters on the branch sampled for starch, the opposite branch, and two branches in a similar tree at the same crown level.

Laboratory procedures

Samples were stored at -10 to -12° C. The samples were then divided into three components; foliage, twigs and bark. Twigs consisted of the xylem left after the bark had been peeled off. The bark was a composite of phloem and rhytidome. The samples were then transfered to vials. During this procedure samples were exposed to room temperatures for two minutes or less.

Samples were prepared for freeze drying by cooling to -40° C. They were then placed in a large (16 cu. ft.) freeze drier and put under a high vacuum (40 microns of Hg) and freeze-dried for four days to ensure adequate drying. When the samples were removed they were capped and immediately put back into the freezer for storage until they were ground and analyzed.

Prior to grinding, all samples were kept in a freezer adjacent to the Wiley mill. The time out of the freezer was limited to under one minute. The samples were ground through a 40 mesh sieve.

After the samples were freeze dried and ground they were allowed to warm to room temperature and a 40 mg. sample weighed for analysis. The procedure used for starch determination was an enzyme technique similar to that of Dekker and Richards (1971) and further developed by Webb and Karchesey[1]. Briefly, the sample is extracted for four hours in a micro-soxhlet with 80 percent ethanol to remove free sugars and phenolics. The starch is extracted with a sodium hydroxide solution, hydrolyzed

1. Webb and Karchesey, unpublished method.

with amylase and amyloglucosidase, and the amount of glucose liberated is determined with glucose oxidase. This reaction is halted with hydrochloric acid which turns the solution purple. The absorbance is then read on a spectrophotometer at 540 nm.; the amount of absorbance is converted to percent starch per unit of dry weight from a standard curve. Sample preparation and analysis times were great therefore, not all samples taken from the tree were prepared and analyzed.

In preparation for electron microscopy, samples were double fixed with glutaraldehyde and osmium tetroxide and dehydrated in a method detailed in Appendix G, but similar to other methods (Meek, 1976; Sjostrand, 1967; Grimstone, 1968; Pease, 1964). The samples were embeded in plastic (Spurr Low-Viscosity Embedding Media), sectioned and stained with uranium and lead. The osmium tetroxide, uranium acetate and lead citrate are all positive stains. Living tissues possess large quantities of atoms with low atomic weights (C, O, N, H). In order to increase contrast or electron interaction, atoms with high numbers must be introduced in a process called positive staining. Osmuim tetroxide is used as a postfix after glutaraldehyde. Tissues fixed in phosphate buffered glutaraldehyde or postfixed in osmium may stain very intensely. Therefore, dilute stains may have to be used. Procedure for fixation is detailed in Appendix H.

Statistical analysis

Satistical analysis was performed on much of the data in various combinations. Since entire populations of trees were not used, an

estimate of differences among trees must be made using sample data. The student's "T" test was used to test for statistical differences between various samples in the study.

"T" tests conducted in this study include:

1. Starch differences between plots at the three crown levels.
2. Starch differences between crowns on each plot, using peak values.
3. Differences between crown levels, as determined by graphic integration of starch values throughout the year.
4. Differences in starch between plots as determined by graphic integration of starch values for the entire year.
5. Growth differences between crown levels on the upper and lower plots.

Scatter diagrams were also used to determine if there were any relationships between two variables of a bivariate population. The following data was graphed in scatter diagrams.

1. An integrated starch value, versus growth for that year.
2. Average starch content versus growth for that year.

Models used for these correlations were both linear and exponential.

RESULTS

Diurnal variation

In all cases studied, the percentage of starch in the bark was much lower than the percentage of starch in the foliage. For example on the June 3 sampling date the percent starch in the upper crown foliage averaged 7.14 percent and the bark 3.75 percent starch. The data presented in Figure 1 has been transformed to expand the scale and accentuate fluctuations, therefore, 4.83 percent actual starch is represented as 100 percent.

Figure 1, Diurnal variation, is a series of overlays intended to aid in the comparison of diurnal variation of starch of the three crown areas. Each sheet represents one level of the corwn. Both the foliage and bark are graphed on a single sheet. It should be pointed out that each point on the graph represents one sample. To view each graph, place a loose sheet of paper below the graph desired.

There are three apparent peaks of starch content in the upper crown during the diel cycle. The first occurs at 8:30 A.M., four hours after sunrise, followed by a midday depression. After this depression starch increases to a high level, about an hour before sunset, before beginning to drop as sunset approaches. The third apparent peak of starch occurs during the early morning hours between 12:30 and 2:30 A.M.

The bark and the foliage levels have fluctuations that follow each other. The fluctuations are strongest in the upper crown where the

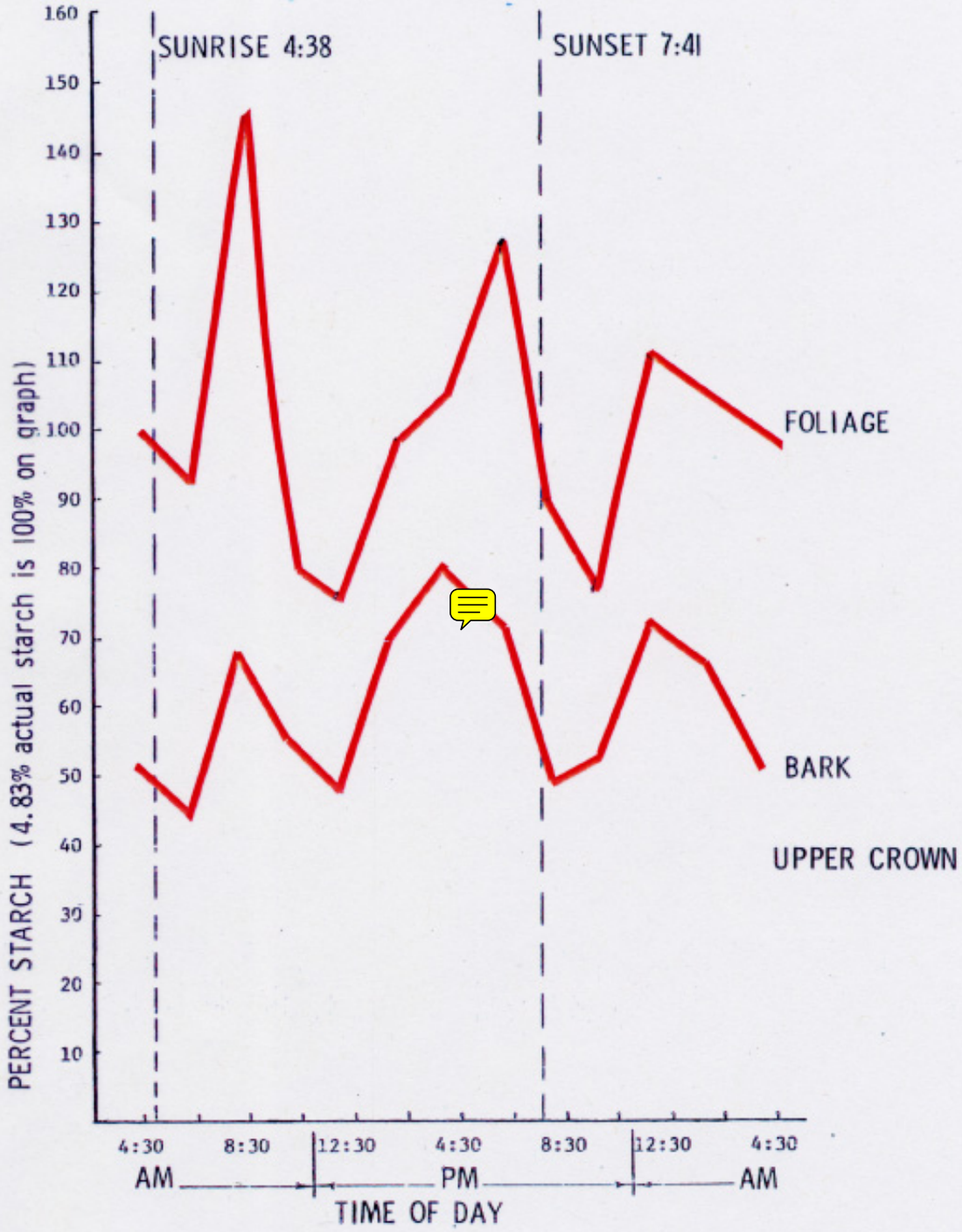

SUNRISE 4:38
SUNSET 7:41
FOLIAGE
BARK
UPPER CROWN
PERCENT STARCH (4.83% actual starch is 100% on graph)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
4:30
8:30
12:30
4:30
8:30
12:30
4:30
AM
PM
AM
TIME OF DAY

FIGURE 1

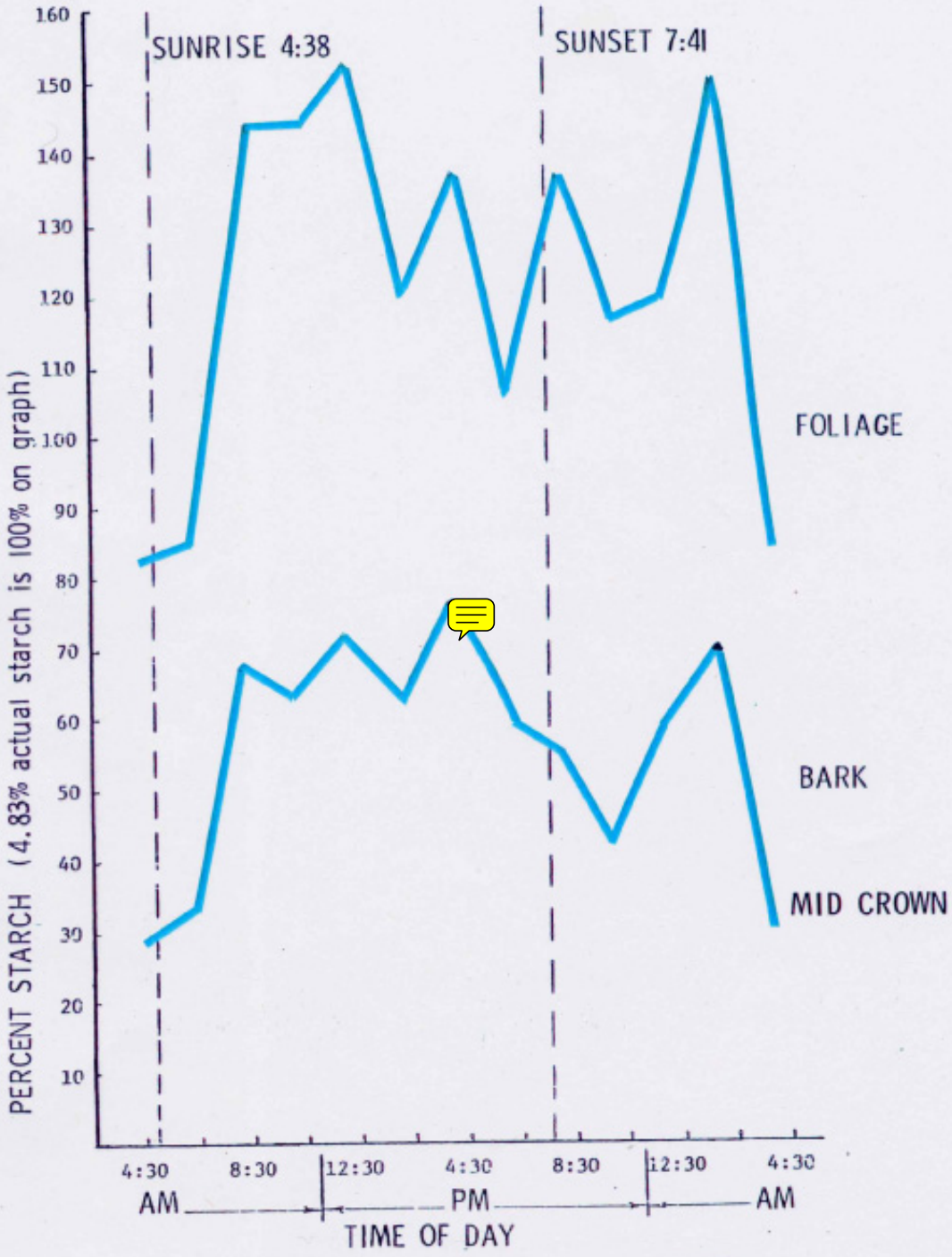

SUNRISE 4:38
SUNSET 7:41
FOLIAGE
BARK
MID CROWN
PERCENT STARCH (4.83% actual starch is 100% on graph)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
4:30
8:30
12:30
4:30
8:30
12:30
4:30
AM
PM
AM
TIME OF DAY

FIGURE I

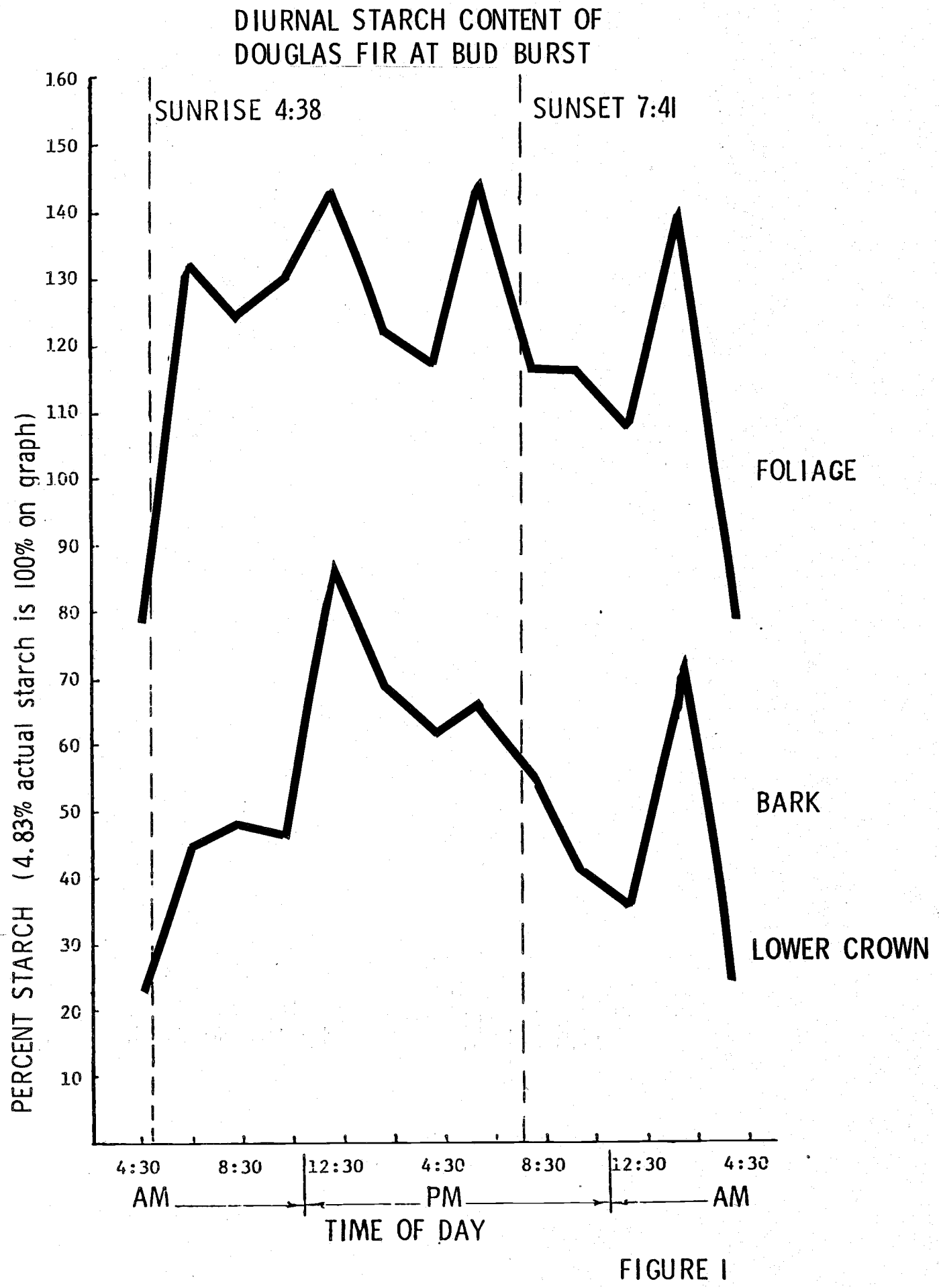

DIURNAL STARCH CONTENT OF
DOUGLAS FIR AT BUD BURST
SUNRISE 4:38
SUNSET 7:41
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
PERCENT STARCH (4.83% actual starch is 100% on graph)
FOLIAGE
BARK
LOWER CROWN
4:30
8:30
12:30
4:30
8:30
12:30
4:30
AM
PM
AM
TIME OF DAY

FIGURE I

DIURNAL STARCH CONTENT OF DOUGLAS FIR AT BUD BURST

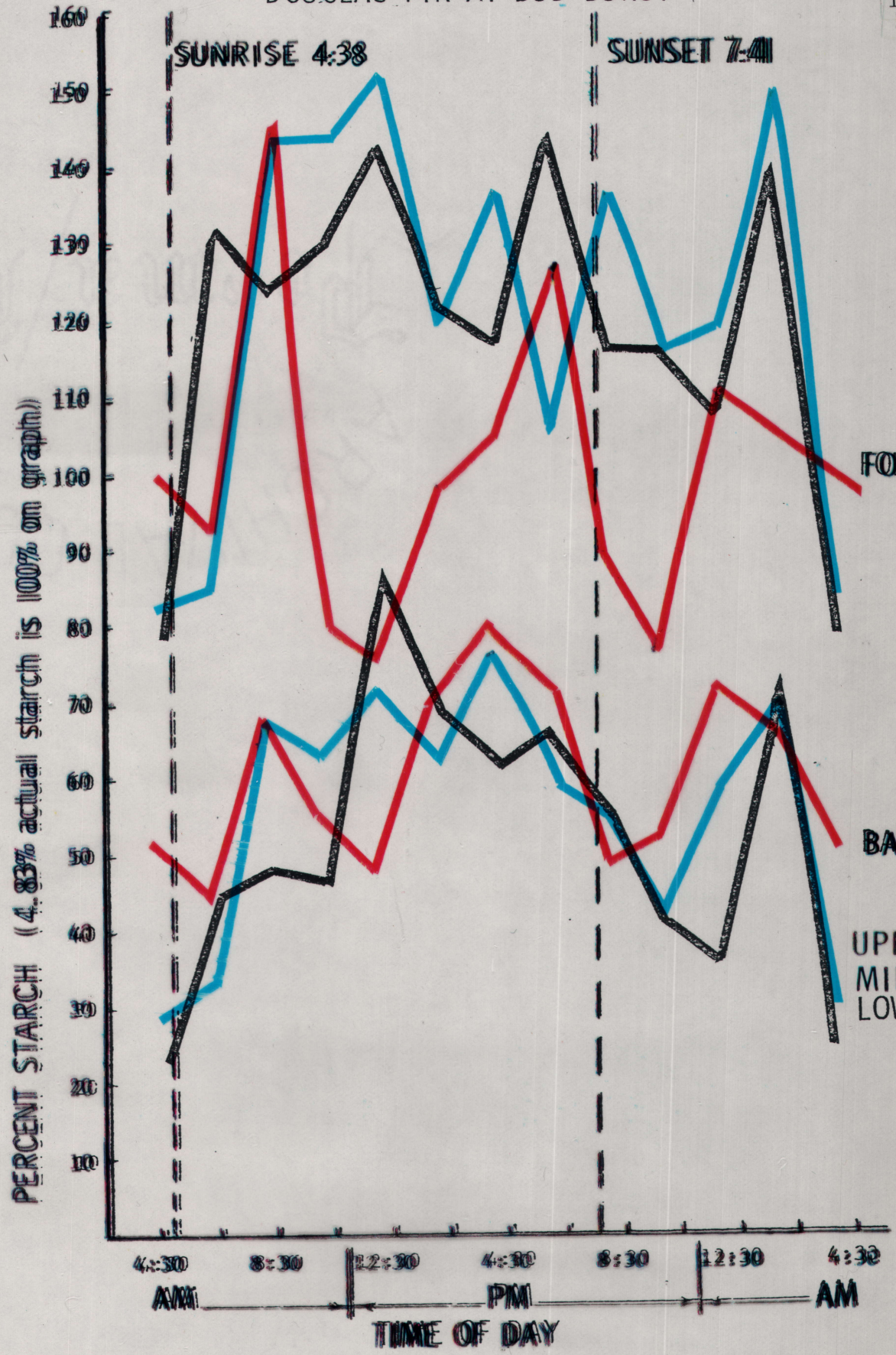


FIGURE I

bark starch content parallels the foliage starch content. The lower crown shows a similar pattern, but the morning and midday peaks are weaker and do not follow each other as closely as in the upper crown (Figure 1). The upper corwn has starch values that flucturate more than the other crown levels, and the foliage has larger relative fluctuations than the bark.

The mid crown starch levels fluctuate at a high level during the day, showing no midday depression. The levels of starch do climb rapidly in the morning but more slowly than the upper and mid crowns. This may be due to the later time at which these components receive sunlight. There is a 2:20 A.M. peak in the starch levels that corresponds to the mid crown early morning peak.

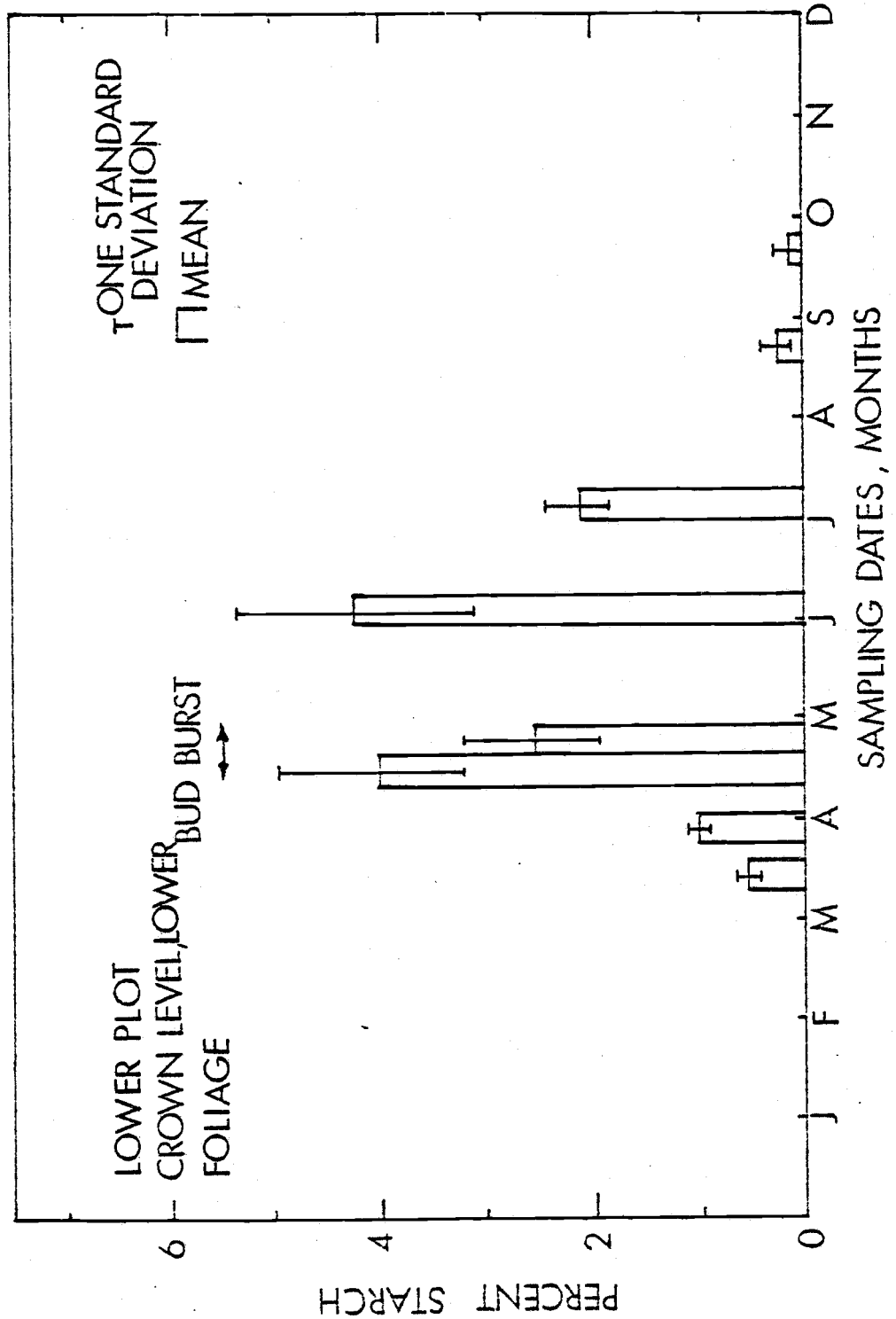
The differences in the peak starch levels of the upper, mid and lower crowns are not great. At sunrise when starch is at its lowest level of the day, the variance in starch content between crown levels is low (+19%). At each crown position the dynamics of the bark starch and the foliage starch are almost identical. As seen in Appendix G there is an early morning peak in starch concentrations in all cases, though at different hours.

Seasonal variation

A large difference in starch content between trees at the same crown level was observed. The lower plot, lower crown and foliage data demonstrate this large between-tree variance in Figure 2. Data for both plots, all three crown levels, bark and foliage, including one standard

FIGURE 2

Seasonal starch variation with means and one standard deviation for lower plot, lower crown foliage.



deviation from the mean is presented in Appendix D. Means have been included here in Figure 3. The dark lines connect the means to indicate the seasonal trends.

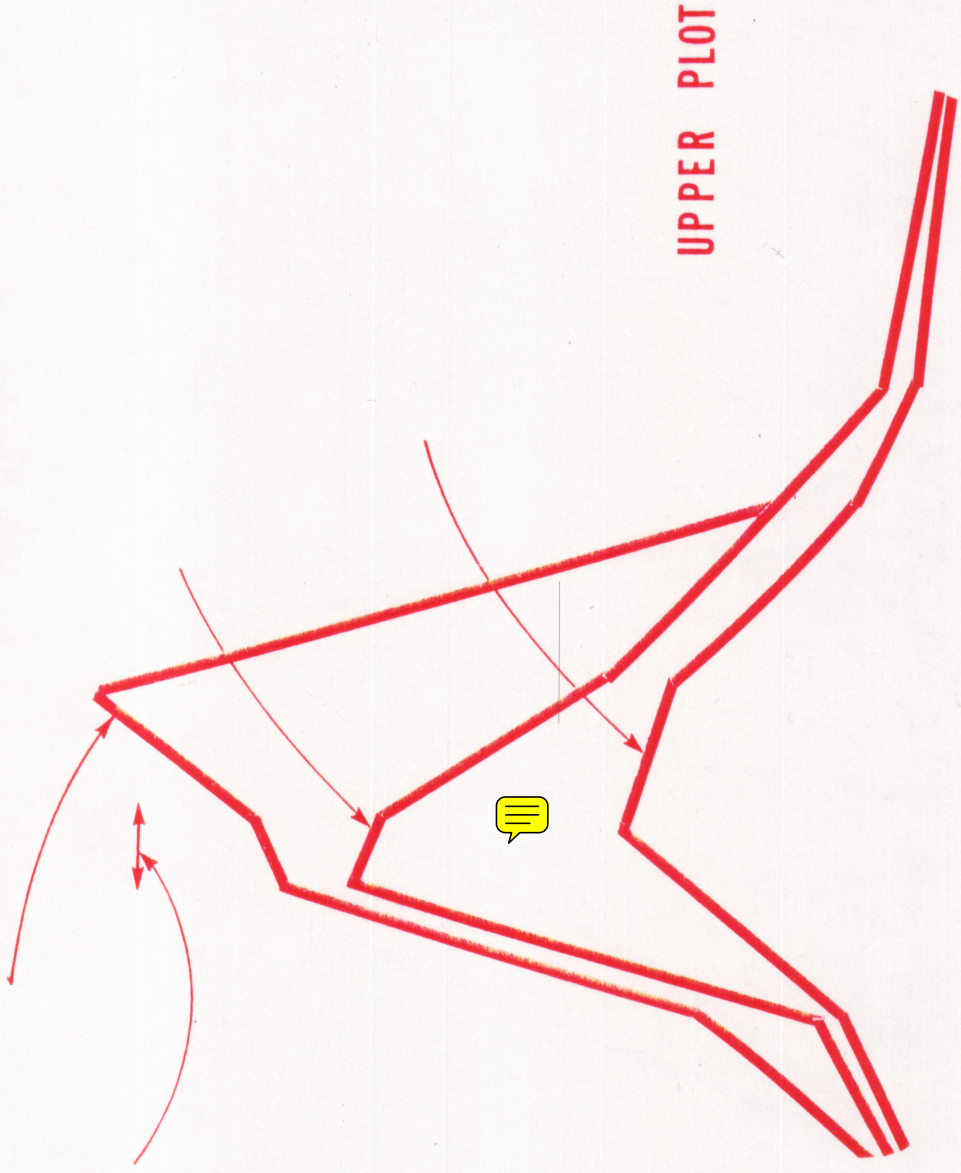
Table (1) includes precipitation data for Corvallis, the watershed which corresponds to the lower plot on Mary's Peak, and the summit, which corresponds to the Mary's Peak upper plot. Corvallis data is presented so that comparisons can be made with the weather data furnished for Corvallis in Appendix A.

Corvallis weather data for each sampling date and the previous date is summarized in Appendix A. The day prior to each sampling date is given since it should have a significant impact on the samples taken at sunrise of the sampling date.

Precipitation occurs primarily in the winter and early spring. The variation in precipitation and radiation due to elevation is dramatic.

Bud burst on the lower plot occurred between April 20 and May 5. Starch rose to a high level in early April and then showed a dramatic decline in mid April. This dip is unexplained by any weather phenomenon that was observed. The starch level then increased to its highest level in early June, followed by a steady and rapid decline. There was no difference in starch levels between the upper and mid crown, but the lower crown has a consistently lower percent starch throughout the year.

The upper plot had a later bud burst than the lower plot, June 1 thru 15. The starch levels did not start to rise rapidly until the beginning of May. This was about one month behind the lower plot.


UPPER PLOT

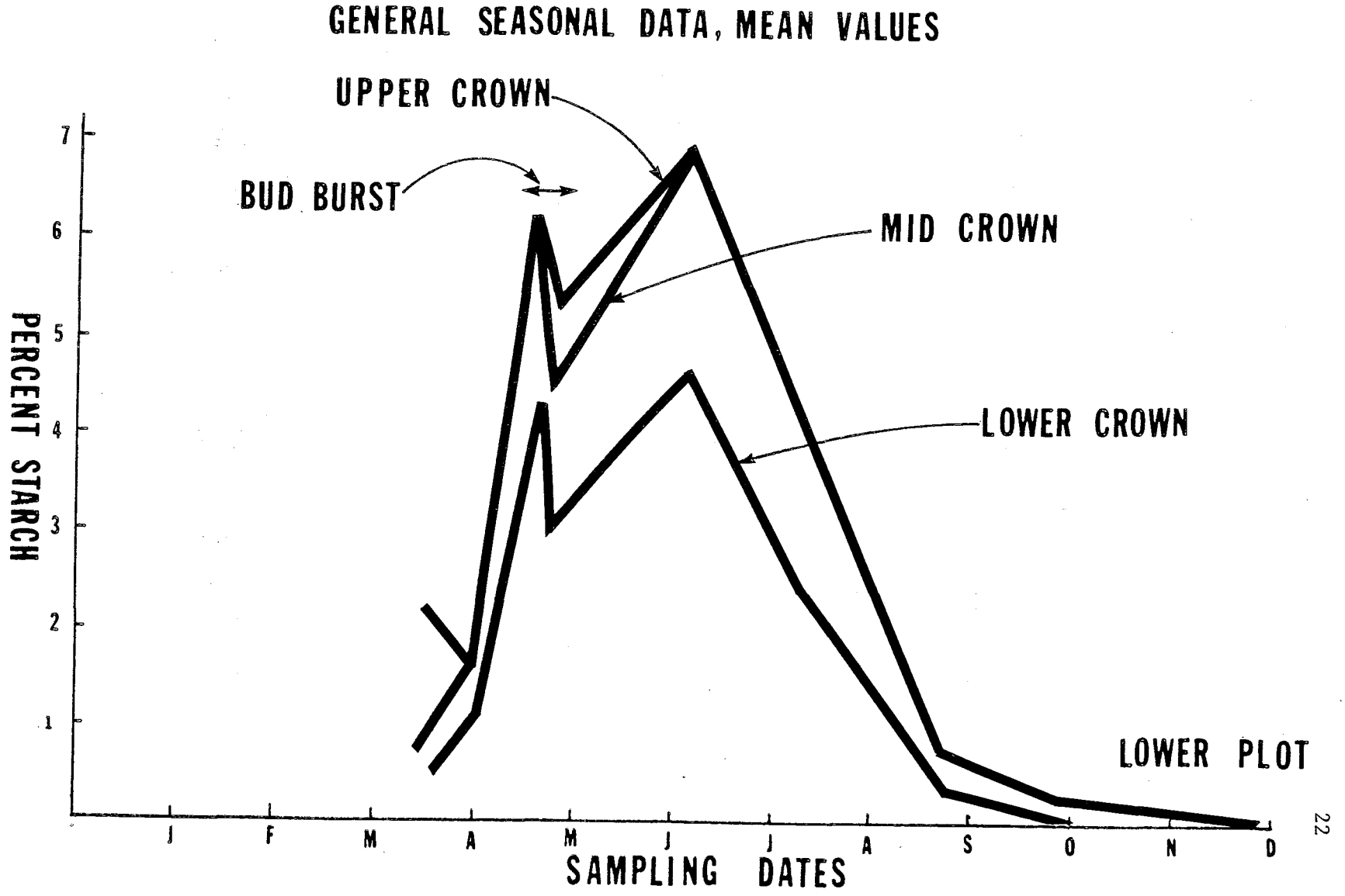

GENERAL SEASONAL DATA, MEAN VALUES
UPPER CROWN
BUD BURST
MID CROWN
LOWER CROWN
LOWER PLOT
PERCENT STARCH
7
6
5
4
3
2
1
J
F
M
A
M
J
J
A
S
O
N
D
SAMPLING DATES

FIGURE 3

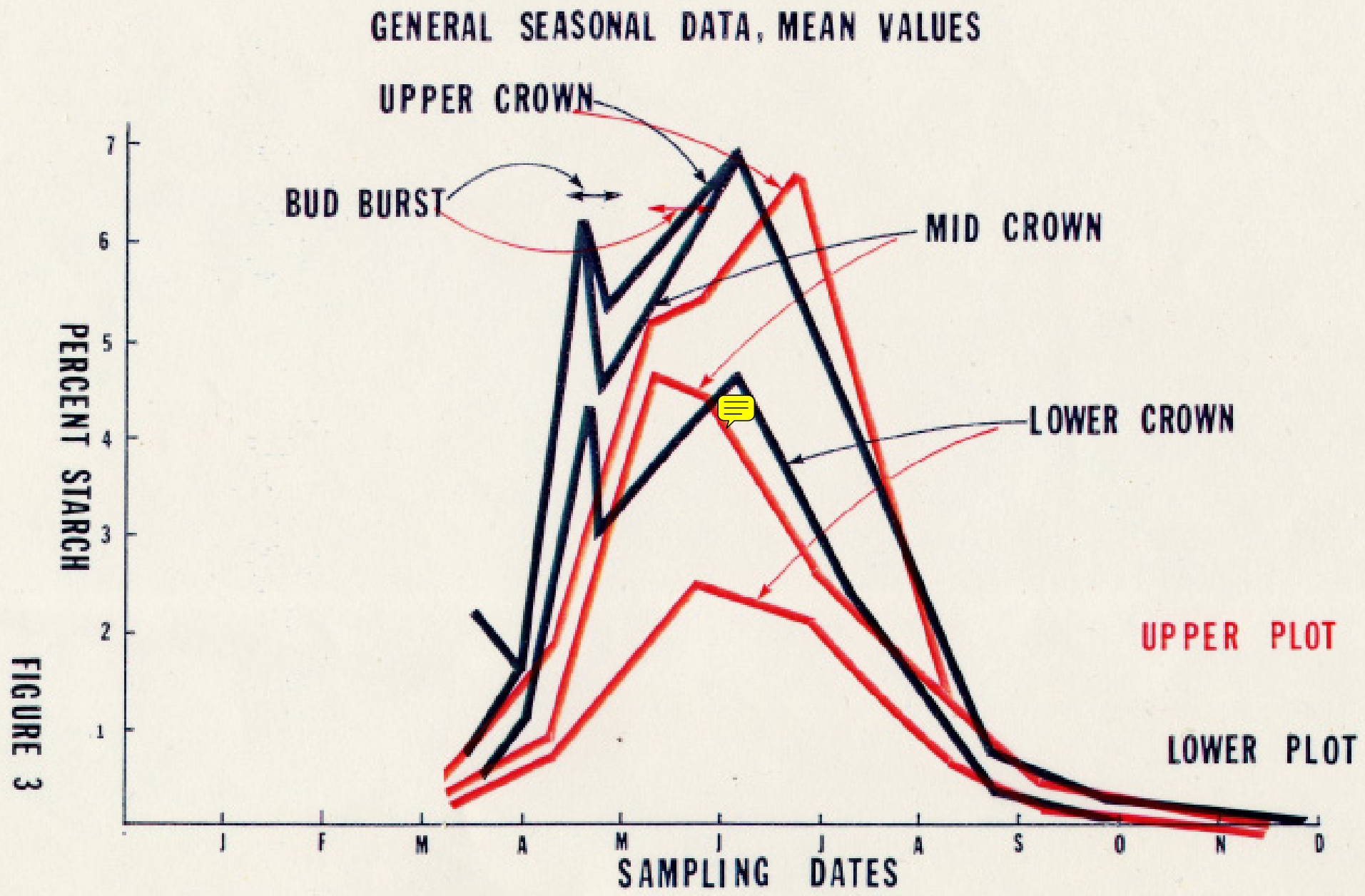

GENERAL SEASONAL DATA, MEAN VALUES
UPPER CROWN
BUD BURST
MID CROWN
LOWER CROWN
UPPER PLOT
LOWER PLOT
PERCENT STARCH
7
6
5
4
3
2
1
J
F
M
A
M
J
J
A
S
O
N
D
SAMPLING DATES

FIGURE 3

TABLE I

General Weather Data for Corvallis, Watershed, and Summit.

Location	Elevation (M)	Avg. Annual Rainfall (cm)	Avg. Annual Snowfall (cm)	Avg. No. Days Receiving .025 cm
Watershed	530	167	--	--
Summit	1160	150	37	160
Corvallis	74	97	19	140

Data obtained from U.S. National Weather Bureau information compiled at Oregon State University.

TABLE 2

Description of plots on Mary's Peak

Plot	Elevation (Meters)	Aspect	Mean Tree Age (Years)	Mean Tree Ht. (Meters)	Date of Bud Burst
Lower Plot	500	SE	25.78	15.0	4/20-5/5
Upper Plot	1128	SE	26.40	9.4	6/1-6/15

Douglas-fir is the dominant vegetation in both plots.

This may be related to the later bud burst of the upper plot. The patterns of starch variation for the upper, mid and lower crown were different from each other. The mid and lower crown reached their highest level of starch before the upper crown. The upper crown did not show peak levels until the middle of July. For details see Appendix F.

The seasonal dynamics of starch are almost identical regardless of plot or crown position, a springtime buildup and a gradual summer decline to an undetectable level in late fall.

Mean starch values indicate that the lower plot has higher starch values than the upper plot, especially at the lower and mid crown levels. The area under the curve for starch produced throughout the year was larger on the lower plot. Because of the small sample size it could not be determined if this difference was statistically significant. With the exception of one tree in the upper plot that had higher starch values in the upper and mid crown than any other tree observed, the starch levels were consistently higher in the lower plot at all crown levels. On both plots starch levels were very low by mid-September and by November no starch could be detected in any of the samples. In the spring the buildup of starch in the upper plot began later. There was snow on the upper plot into the month of June, whereas the lower plot only had occasional traces of snow throughout the winter. Differences in solar radiation between elevations are expected, but cannot be predicted (Holbo, 1977).[2] More total as well as short wave radiation

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2. Holbo, Forest Research Lab, Oregon State University, personal Communication.

is expected at higher elevations, this being due to less filtering by airborne particulates. Temperatures are expected to be lower at higher elevations.

Starch levels were examined using a students "T" test for differences between plots using peak starch values and no differences could be determined. An integrated value of starch content for each tree throughout the year was created by taking the area under a tree throughout the year was created by taking the area under a curve connectiong the means of starch values for each sampling date. Using this integrated value a "T" test was performed and no significant differences between plots were found.

Differences in starch levels between crown levels were expected, using "T" tests these variations were tested. Peak values for starch showed no differences among crown levels on each plot. The integrated values did show a significant difference on both plots, 95 percent confidence, between the mid and lower crown, but not between the upper and mid crowns. Generally, mean values for starch are lower in the lower crown, although the statistical tests were not definitive.

Growth differences

Differences in the total height of the trees on the two plots, were observed. On the upper plot the mean height of the trees was 9.4 meters and the mean age was 26.4 years; the lower plot mean tree height was 15 meters and mean age is 24.8 years. Thus it was assumed that there was a difference in growth of the trees of both plots. Growth of lateral branches did not differ between the two plots at any crown level during

TABLE 3

Mean Growth Data For Branches at Three Crown Levels in Two Stands of Douglas-Fir for 1975, 1976

		Upper Crown		Mid Crown		Lower Crown	
		Mean Growth (cm)	s	Mean Growth (cm)	s	Mean Growth (cm)	s
1975	Lower Plot	21.95	1.33	13.31	1.25	7.84	0.87
	Upper Plot	22.35	2.19	15.92	0.89	8.82	0.92
1976	Lower Plot	21.63	1.10	11.51	1.49	7.83	1.35
	Upper Plot	22.12	2.68	9.80	1.50	7.23	1.17

either 1975 or 1976 (Table 3). The differences in total height was due to the breaking of the tops of the trees on the upper plot by severe winter weather associated with its higher elevation and more exposed position near the crest of a ridge.

There were differences in growth between the three crown levels. The upper crown averaged 22 cm. a year, the mid 12.6 cm., and the lower 7.93 cm. In the upper and lower crowns no differences in growth could be seen between 1975 and 1976. The mid crown showed about 30% less growth in 1976 than 1975. There was very little difference in growth between the upper plot and the lower plot either year.

A series of "T" tests were used to examine differences in growth on each plot, and on combined plot data, between the upper, mid and lower crowns to determine significance. At the 95 percent conficence level there were no significant differences in growth between crown classes. On the lower plot differences between the upper and mid crown was observed, but only at the 80 percent conficence level.

Starch, Growth Relationships

It was hypothesized that the growth of a branch is related to the amount of starch produced by that branch. Within each crown level, branch growth was compared with both peak starch values and integrated starch values. The integrated starch value and mean growth of the sample branches show a curvelinear pattern when graphed. Also the data formed clusters by crown level. Lower crown branches had both a low starch value and a small amount of mean growth. Mid-crown branches had a high starch value and a small mean growth. Upper-crown branches showed high

starch values and large mean growth (Figure 4). The clustering of the data by crown level indicates that both crown and level can be predicted if one knows starch content and growth. Integrated starch level, however, cannot be predicted from branch growth.

For the purpose of this study the crown was arbitrarilly divided into three levels. It could just as easily have been divided into only two levels, upper and lower. If the data is looked at in this manner, using the upper and lower crown level data to represent this bilateral division, a definite trend is seen. Branches in the upper crown consistently show a greater mean growth and a higher integrated starch level than branches in the lower crown.

Starch granule

Starch has been shown to exist in the form of granules for many plants (Banks and Greenwood, 1975). Previous studies done on starch in Douglas-fir have relied upon chemical analysis to indicate the presence of starch. In this study an electron microscope was used to observe the chloroplasts in the cells of the needles of Douglas-fir. Starch appears as large spherical bodies located among the grana in chloroplasts. These bodies were observed in Douglas-fir and several micrographs were taken. The following micrographs are included to show these starch granules. Micrographs of cells fixed at 6:30 A.M. and 6:30 P.M. were obtained. The contrast between the two times is marked. The 6:30 P.M. chloroplasts show dark staining bodies or lipid droplets, whereas the 6:30 A.M. do not have these. The other easily seen differences is the folding of

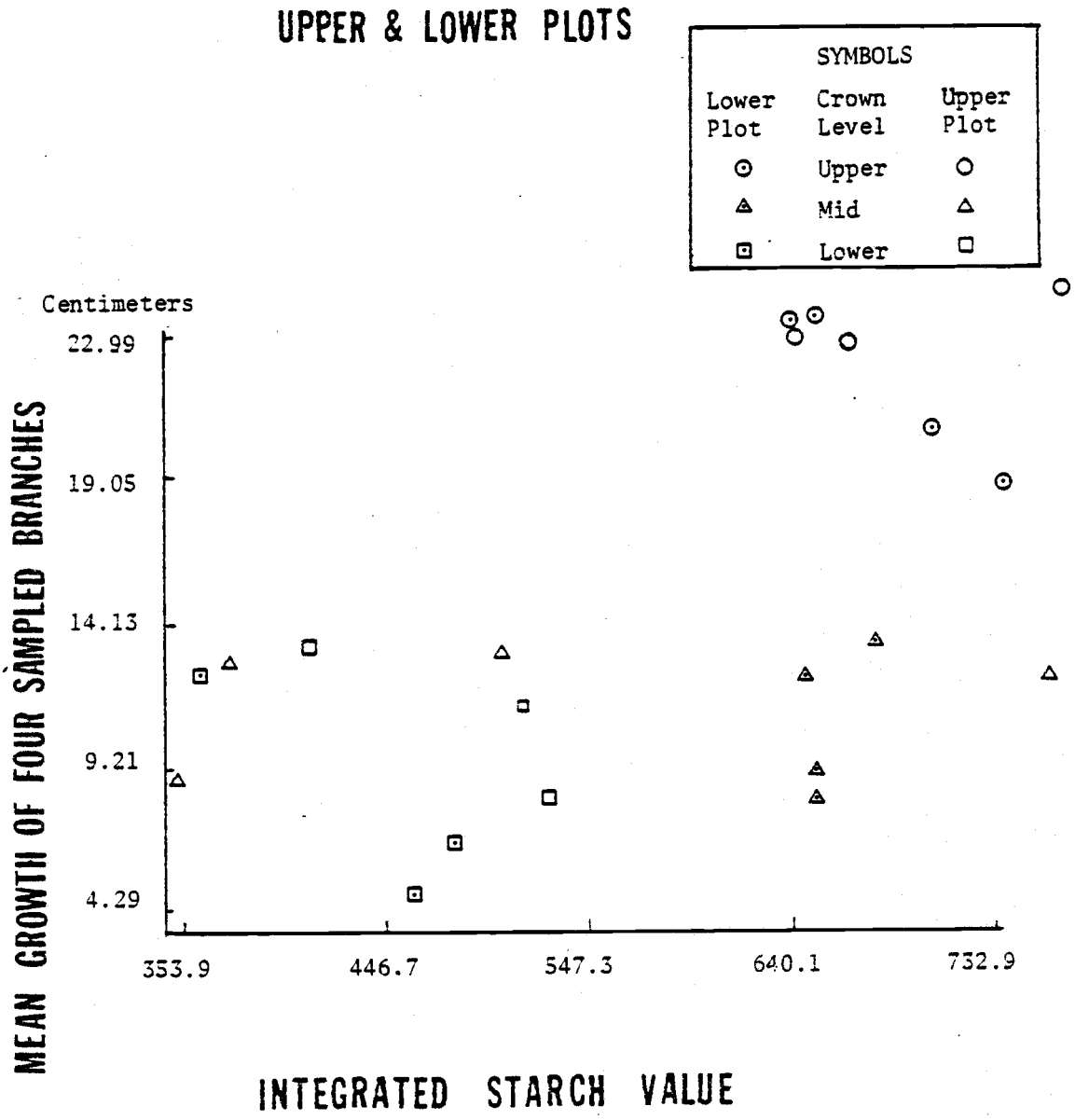

Figure 4: This graph shows the separation of the samples by crown level in a scatter between growth and starch.

the starch granule in the 6:30 A.M. micrographs. This folding may either be due to shrinking of the granule overnight or folding due to stress placed on the sample during the thin sectioning of the material. It should be noted that these were the only micrographs taken therefore interpretation is not definitive.

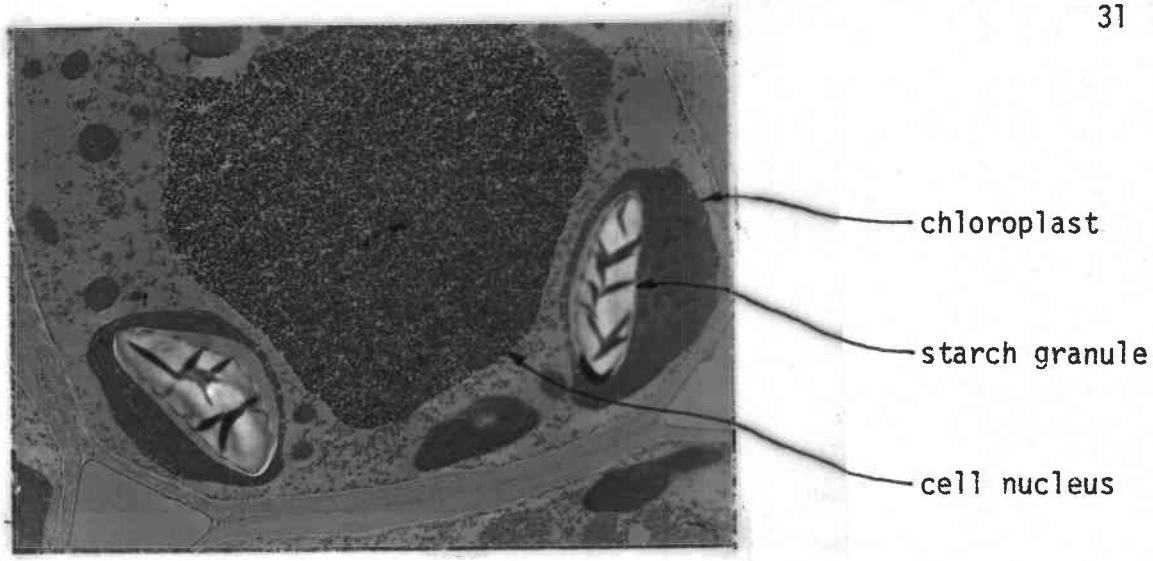

Figure 5. Cell of Douglas-fir needle magnified 6,400x. This material was fixed at 6:30 AM.

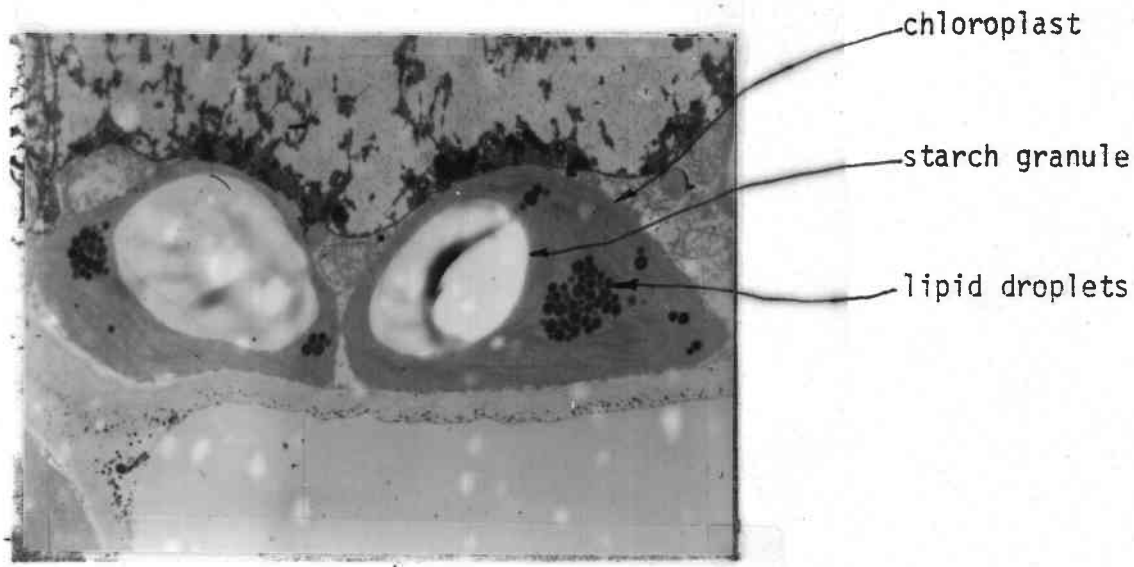

Figure 6. Cell of Douglas-fir needle magnified 9,500x. This material was fixed at 6:30 PM.

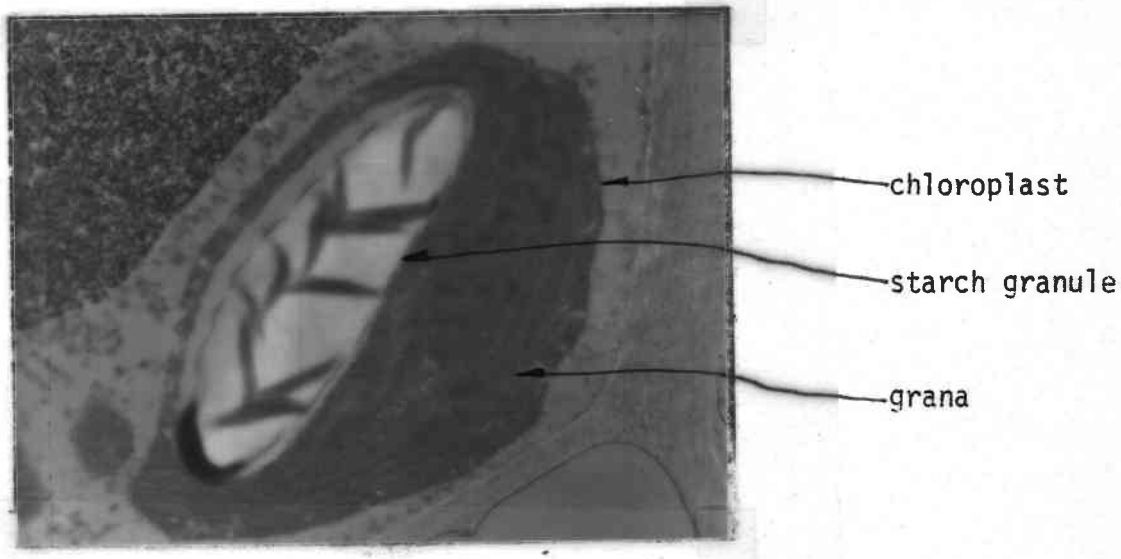

Figure 7. Chloroplast of a Douglas-fir needle magnified 15,200x. This chloroplast was fixed at 6:30 AM.

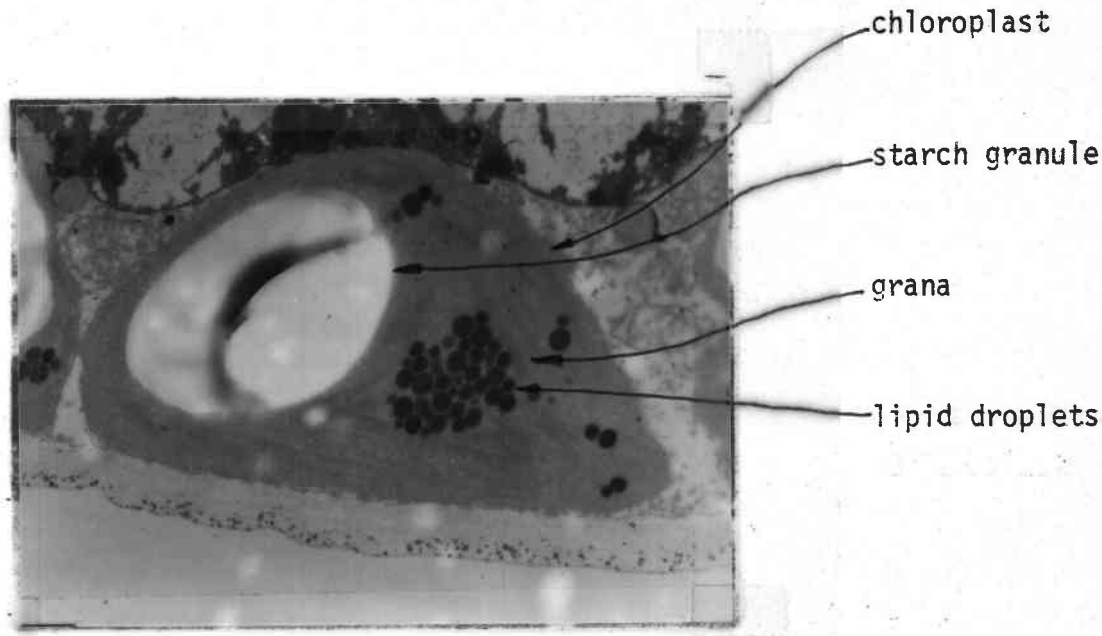

Figure 8. Chloroplast of a Douglas-fir needle magnified 15,200x. The chloroplast was fixed at 6:30 PM.

DISCUSSION

In studies of starch analysis seedlings have been used, and while seedlings overcome some of the technical problems of sampling large trees, they are subject to other errors. For example, seedling material has been shown to possess a photosynthetic capacity which may be considerably greater than that of mature foliage (Hodges, 1962). The present study used older trees to obtain data that is more usable.

In both the diurnal study and the seasonal study the bark showed a consistently lower percent starch than the foliage. This was due to the nature of the samples not the method of sample preparation. The bark was removed from the stem as a single unit, this being composed of two major parts, the live phloem and the dead rhytidome. The starch is produced in the leucoplasts located in the live phloem and is not present in the rhytidome. Therefore, when the bark is analyzed for starch content, the amount of starch per unit of dry weight is reduced. The foliage, on the other hand, is composed largely of live material.

Diurnal variation

The starch content in both the foliage and bark of a connected twig climbed rapidly in the first hours after sunrise. This is probably the result of an increase in the photosynthesis rate and the concomitant increase in assimilates. Immediately after sunrise, the rates of photosynthesis climb very rapidly (Polster, 1950, Ah-Sing Chia Looi and

Cummings, 1972; Pallas et. al., 1974). The starch levels then drop indicating a possible midday depression of photosynthesis.

A midday depression in photosynthesis has been reported by (Helms, 1965; Kramer and Kozlowski, 1960; Polster, 1950). Though the midday depression in the starch levels in both the foliage and bark has not been reported, it was not entirely surprising considering the close connection between photosynthesis and starch production. Present theory generally indicates that the midday depression in photosynthesis may be caused by increasing water stress (Brix, 1962). A possible third peak in starch content occurs between 12:30 and 2:30 A.M. With only one sample at each point statistical analysis is impossible. This peak is very interesting and has not been mentioned in any of the literature reviewed.

The plant produces starch in the chloroplasts of the mesophyll cells in the needles. This starch must be converted to sucrose for transport and then reconverted back to starch in the phloem protion of the bark. Much work has been done on the enzymatic mechanism involved in this interconversion, but some of the actual relationships remain unclear. The biochemical relationships involved in this interconversion process are extensive and are not covered here as they go beyond the scope of this paper. For a good review of the subject see Akazawa (1976).

The apparent absence of a lag time for translocation of photosynthate from the foliage to the bark in this study differs with the findings of Mason and Maskell (1928). They reported a lag time between the diel fluctuations of sugars in the foliage and in the bark. This

creats a dilemma since it is the sugars not the starch that are translocated. The diel fluctuations in sugars should be very similar to the fluctuations in starch. The translocation of nutrients occurs at rates from 10-15 centimeters per hour with higher rates occasionally reported (Reiner, 1960). This would indicate that sugar is translocated rapidly as well. The sampling time in this study of two hours would miss this rapid translocation.

Seasonal variation

The results presented in this paper for seasonal variation of starch are very similar to those of Kruger and Trappe (1967) and Little (1970a, 1970b). However, Kruger and Trappe's (1967) study was limited to seedlings of Douglas-fir. This omits the problems of sampling associated with more mature stands of Douglas-fir. Another sampling problem that occured was the fact that Kruger and Trappe sampled in what they called "late morning" it would seem that there are two problems with this classification. In the first place "late morning" is not a specific time. Secondly, should one find "late morning" an acceptable term, then this would seem to be a poor time to sample for diurnal variation. The best time of day to sample would be within an hour before sunrise. This sampling time avoids the bias created by reduced photosynthesis in cloudy weather as well as the reverse condition. Similarly, Little (1970a) used more mature balsam fir, but sampled the trees at an unspecified time creating problems again in interpreting this data.

The present study sampled 35-40 year old, young growth Douglas-fir before sunrise. By beginning the sampling process before sunrise one can minimize the effects of weather conditions the previous day.

Starch concentration in both the foliage and the bark began to increase just prior to the time of bud burst. The starch concentration rising earlier in the lower plot correlated with an earlier bud burst time while a later increase in starch concentration correlated with a later bud burst in the upper plot. Little (1970a), claims that most of the springtime increase in starch is derived from current photosynthesis. This build-up in starch production is also correlated with increased in growth of roots prior to bud burst (Shiroya et al., 1962, 1966). The findings of Lavender et al (1970) suggest that the activity of root systems in Douglas-fir seedlings is dependent upon materials exported from the shoot. The growth increase in the roots prior to bud burst requires energy in the form of carbohydrates, transported from the needles. Using a girdling technique and detecting a build-up of starch above the girdle, (Little, 1970b) suggests that current photosynthates are exported early in the season. The springtime increase in carbohydrates has been observed in the needles and bark in numerous conifer species (Hepting, 1945; Kruger and Trappe, 1967; Kimura, 1969; Jones and Steinacker, 1951 and others). The build-up of starch prior to bud burst is more complex than a temperature mediated conversion of sugar to starch as proposed by Hilliard (1970) for average daily temperatures did not begin to rise until the beginning of May. This is well after the dramatic rise in the starch levels in the lower plot.

Most of the starch accumulated during the spring appears to be derived from current photosynthesis. After this springtime accumulation of starch, there is a dramatic decline. This may be due to rapid shoot elongation following bud burst in early spring (Kruger and Trappe, 1967). Starch levels fluctuate dramatically by season, and thus appear to be a readily available food reserve. The fact that starch reserves are not detectable during the winter is an indication that in Douglas-fir, starch in the components studied is not a long term storage pool for energy. It should be noted that the roots were not sampled in this study.

Many of the differences in starch levels between crown classes and plots that were expected were not observed. This was due to the large differences between the individual trees sampled in each of the crown classes. Data for variation between trees on the same plot could not be found in the literature. Mose of the previous literature on starch variation lists a series of numbers without mention of ranges or standard deviations (Kruger and Trappe, 1967; Little, 1970a, 1970b). Variation in the starch content of open grown trees is small, (Webb and Kilpatrick, 1976).

In general, light levels down through the crown vary (Kira, 1975; Saeki, 1975), with the highest amount of light at the upper surface, an intermediate value in the mid crown, and least amount of light through photosynthesis, starch levels would appear lower in the lower crown and highest in the upper crown. In the area studied the mean values of starch support this interpretation. In fact the lower crown consistently synthesized the least, the upper and middle crown levels seemed to share

in the production of the higher starch level. But the statistical differences are not significant, therefore this interpretation is not conclusive.

CO_2 profiles within a forest canopy exist; in a spruce forest (Baumgartnen, 1969), in a pine forest (Denmead, 1969), and in a tropical forest (Lemon, Allen and Muller, 1970). During daylight hours these profiles are not great, however, one is still able to recognize a higher CO_2 level in the lower canopy than the middle and upper regions. This higher CO_2 level in the lower crown may aid in photosynthesis.

The patterns observed in the scatter diagram of points correlating growth and percent starch should be investigated further. In figure (4) lower starch correlated with lower growth in the lower crown while more starch correlated with higher growth in the upper crown. Inconsistent with these findings the mid crown showed a low growth, high starch relationship thus damaging a direct correlation there may have been between starch and growth.

SUMMARY AND CONCLUSIONS

The results of this study show that this method of starch analysis is a powerful tool in investigating energy dynamics in Douglas-fir. This study has uncovered many new concepts and posed problems of sampling young conifer stands that should be investigated in future studies.

The diurnal pattern of starch synthesis and depletion is very interesting and has not been reported by others; a similar experiment using a larger sample size and several diurnal cycles at different times of the year could prove useful. The diurnal pattern reported in this paper should be an aid to future investigators in the area of starch reserves as it indicates that the time of day, in relation to sunrise, that samples are taken is crucial. In particular the A.M. peak in starch concentration needs to be investigated. An osmoregulation theory was proposed in this paper, but data on sugars would be needed to confirm it. Levels of sugar as well as other possible solutes should be investigated.

The large between tree variation presented in this paper has not been reported by others and since this was the major contributing factor in the inconclusive results, it should be explored thoroughly. The pattern of data presented here on correlation between starch and growth was interesting. An attempt to improve this should be made using more areas of the crown to smooth out the curve. Another growth variable might be biomass, as opposed to elongation.

The findings represented in this paper have greatly increased our understanding of energy reserves in Douglas-fir. The possibilities for new avenues of research into this area have been clearly delineated.

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APPENDICES

APPENDIX A

Weather data for the Corvallis area for 1976

Date	Sampling Points	Temperature max.	min.	Precipitation	Solar Radiation in Langleys
3/14		51	40	.41	144
3/15	X	55	37	T	204
3/16		59	41	0	192
3/17	X	65	46	0	216
3/30		59	36	0	204
3/31	X	61	37	.56	168
4/20		52	44	.36	264
4/21	X	55	34	T	264
4/26		54	36	.01	200
4/27	X	58	39	T	220
4/28	X	70	42	0	204
5/21		67	37	0	540
5/22	X	73	41	0	372
6/2		57	34	.04	528
6/3	X	62	35	T	492
7/8		71	54	.35	560
7/9	X	74	54	0	550
8/19		79	47	0	300
8/20	X	75	52	0	450
9/19		72	45	0	350
9/20	X	87	54	0	300
11/29		46	23	0	100
11/30	X	46	23	0	100

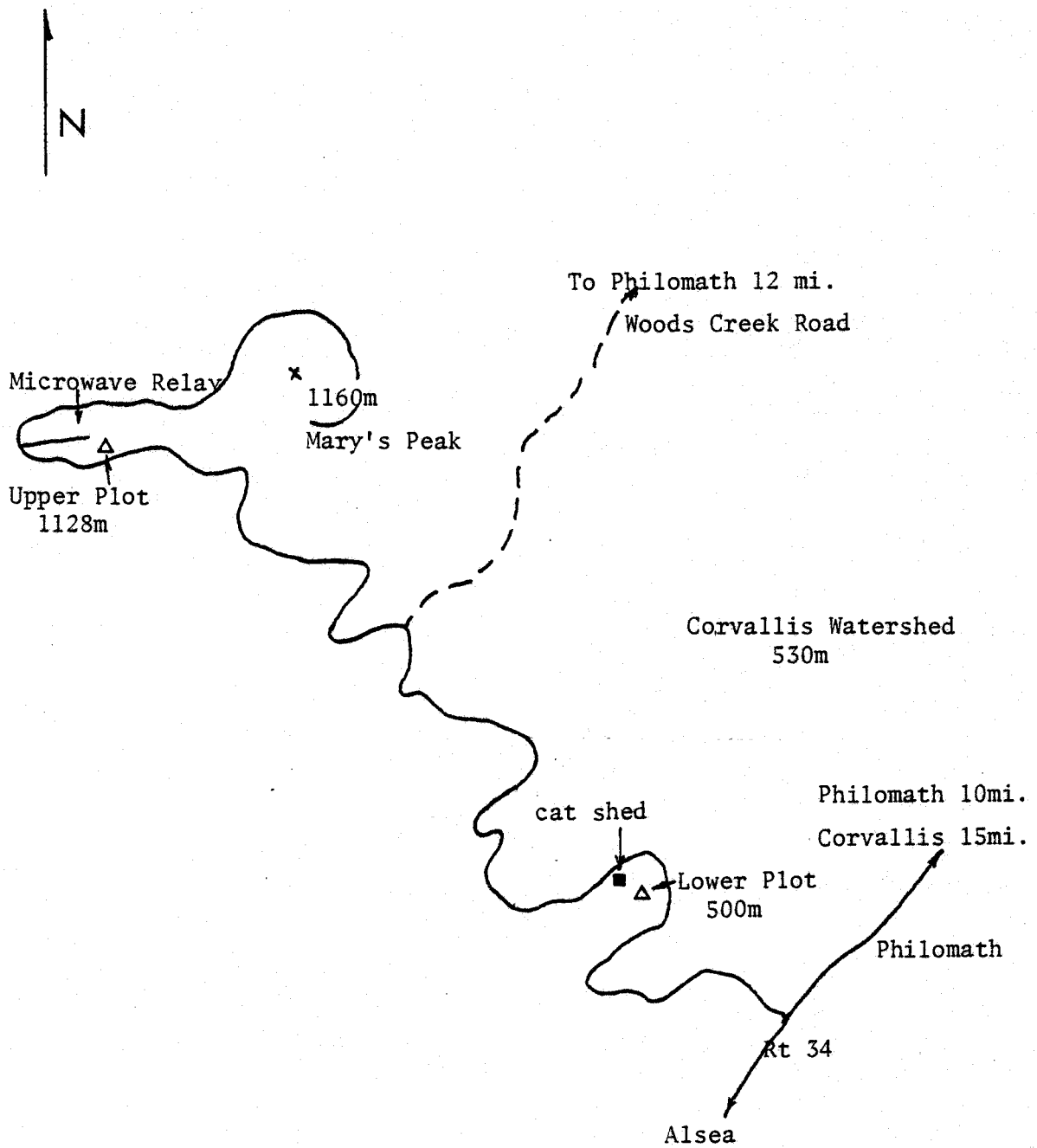
APPENDIX B

	M.P.U.		M.P.L.	
	Height Meters	Age Years	Height Meters	Age Years
DF 1	9.7	24	16.0	27
DF 2	9.0	25	15.5	25
DF 3	9.0	28	15.0	28
DF 4	10.0	25	14.5	24
DF 5	8.7	26	14.3	24
DF 6	9.3	26	13.3	25
DF 7	10.3	28	13.7	25
DF 8	9.3	28	13.7	26
DF 9	9.7	28	16.3	25
DF 10	8.7	26	16.0	26
Mean	9.4	26.40	15.0	25.78

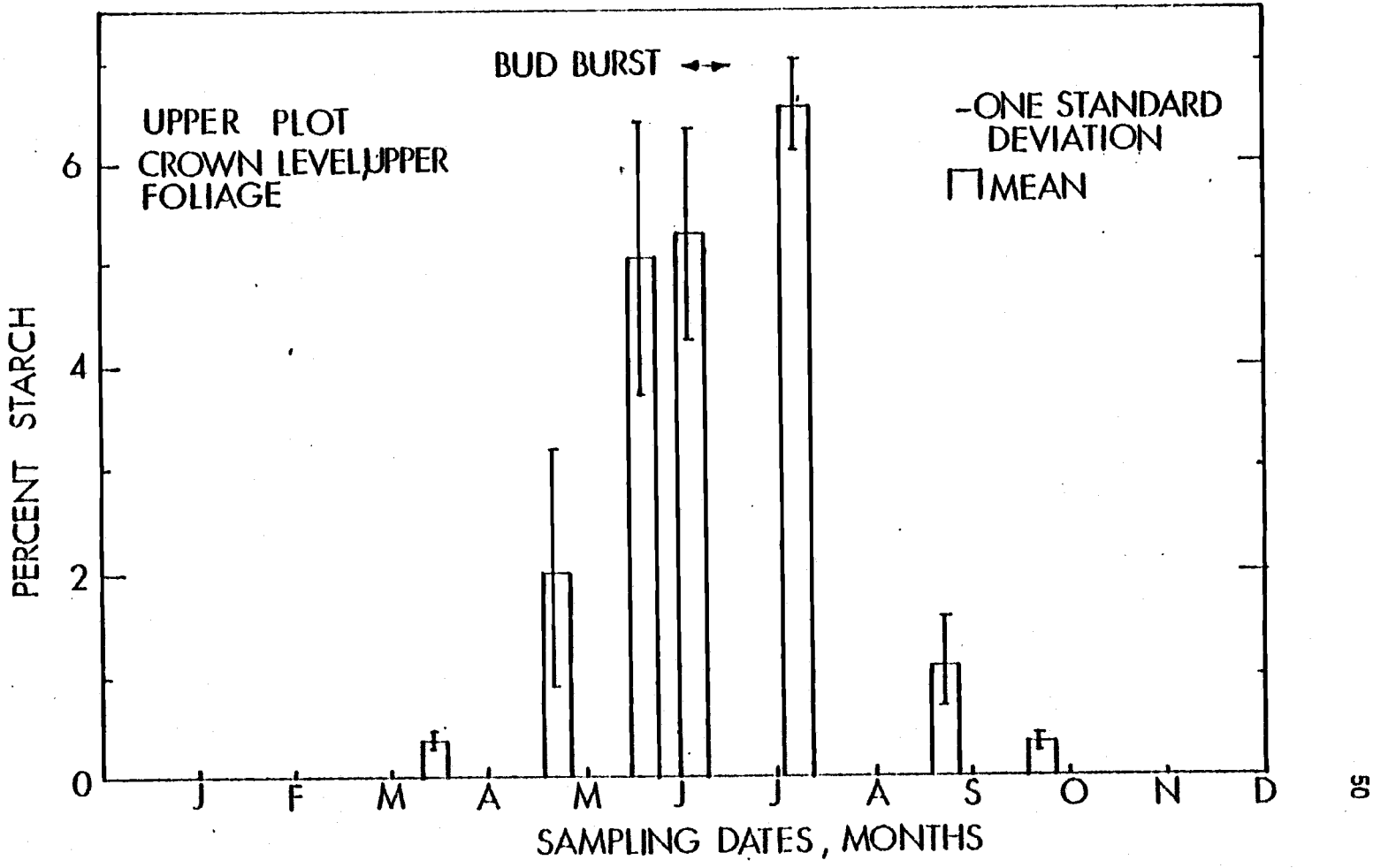
Comparison of average height and age of Douglas-fir (DF) at two test sites Mary's Peak Upper (M.P.U.) and Mary's Peak Lower (M.P.L.).

APPENDIX C

Map showing the two plots used for the seasonal variation of starch in Douglas-fir.



APPENDIX D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UPPER PLOT
CROWN LEVEL UPPER FOLIAGE
-ONE STANDARD DEVIATION
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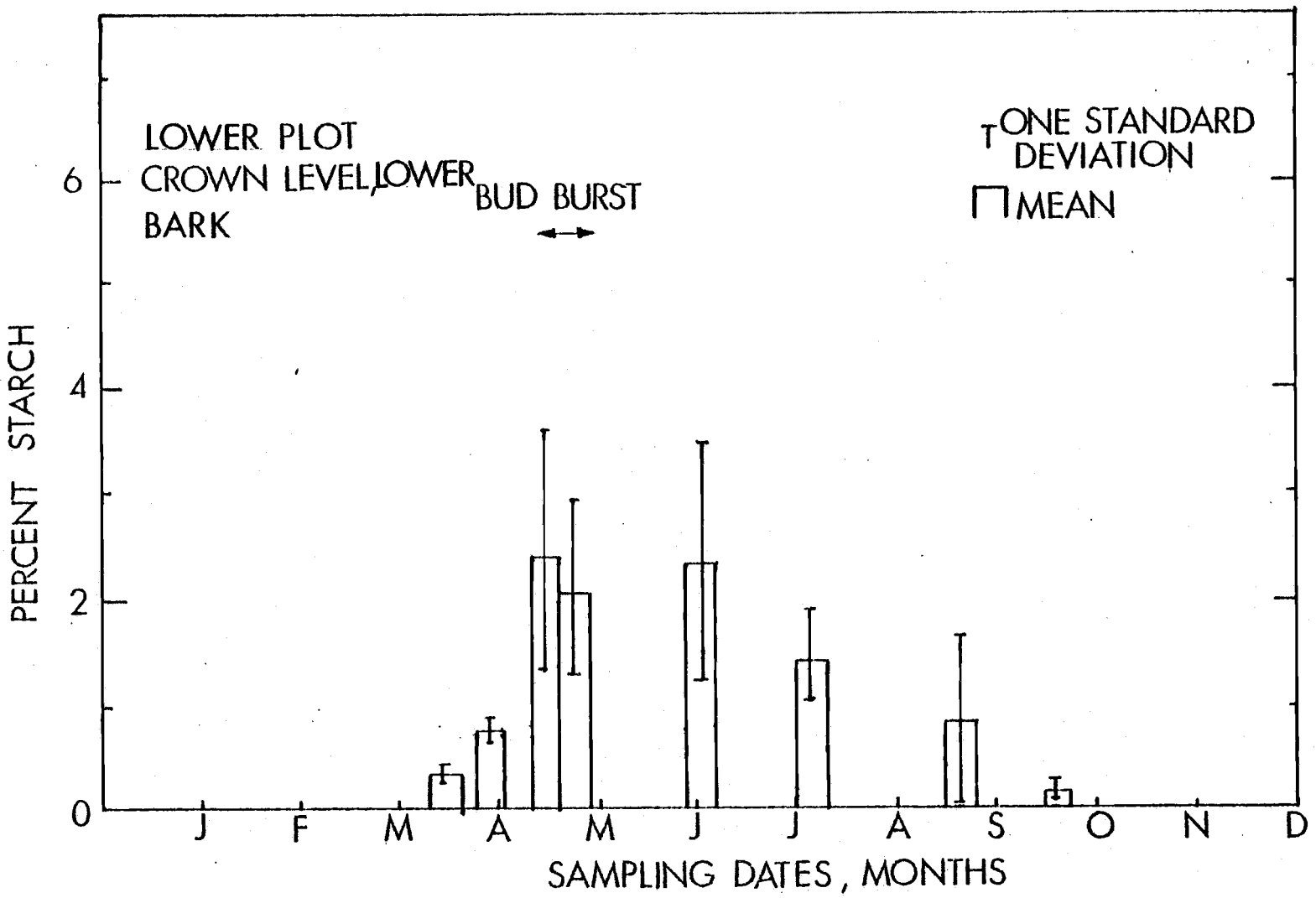
SAMPLING DATES, MONTHS

APPENDIX D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CROWN LEVEL, LOWER BARK
BUD BURST
ONE STANDARD DEVIATION
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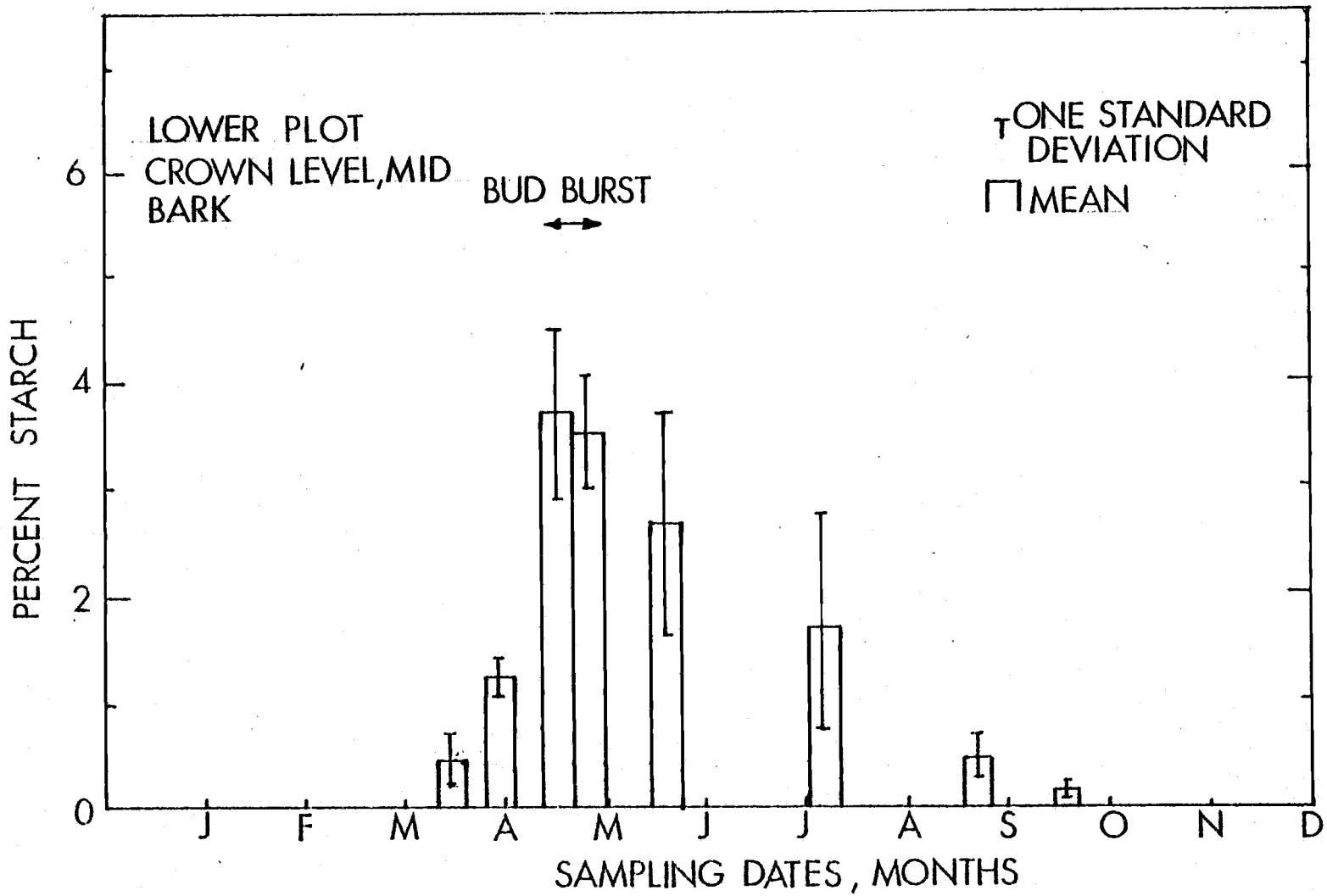
SAMPLING DATES, MONTHS

APPENDIX D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CROWN LEVEL, MID BARK
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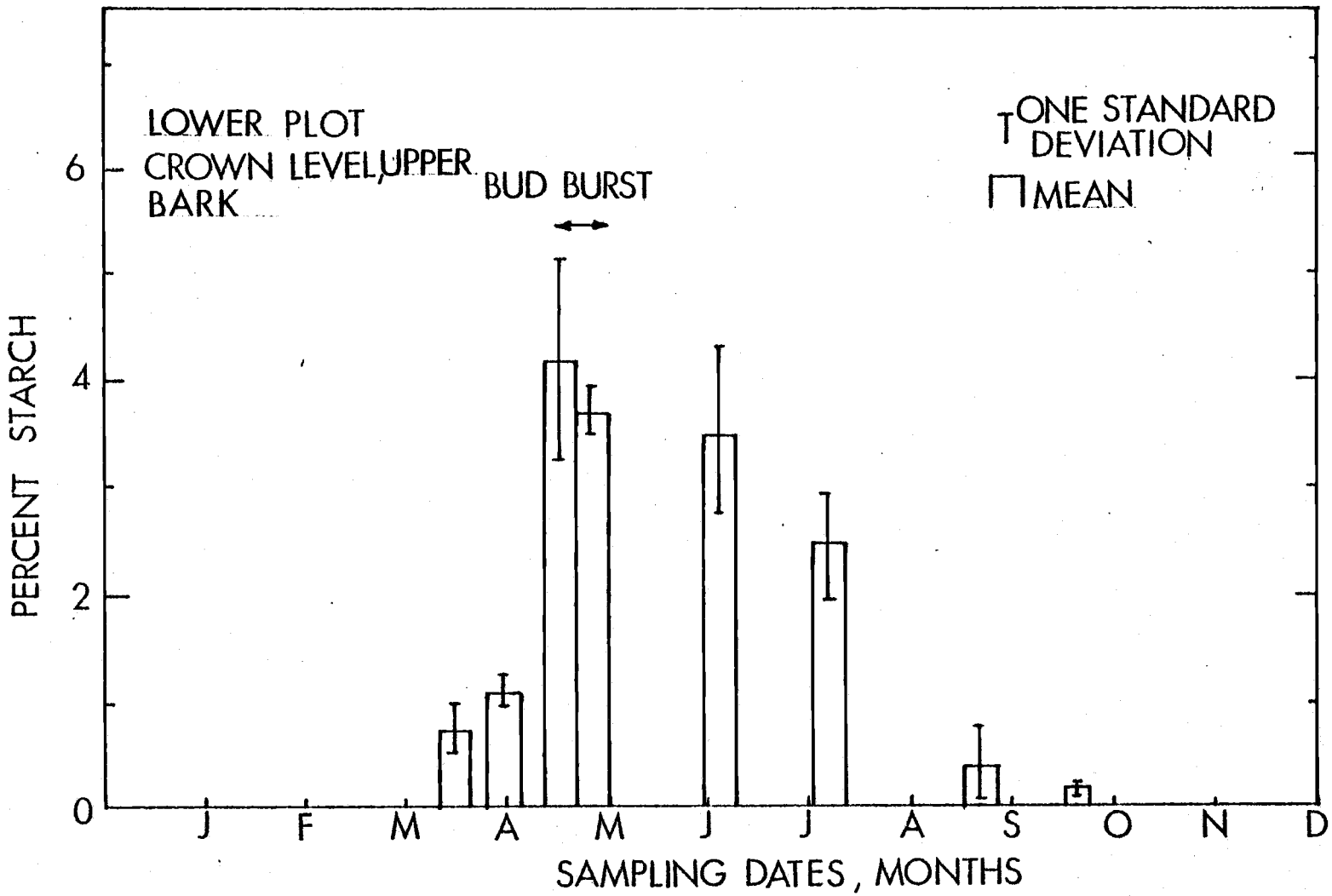
SAMPLING DATES, MONTHS

APPENDIX D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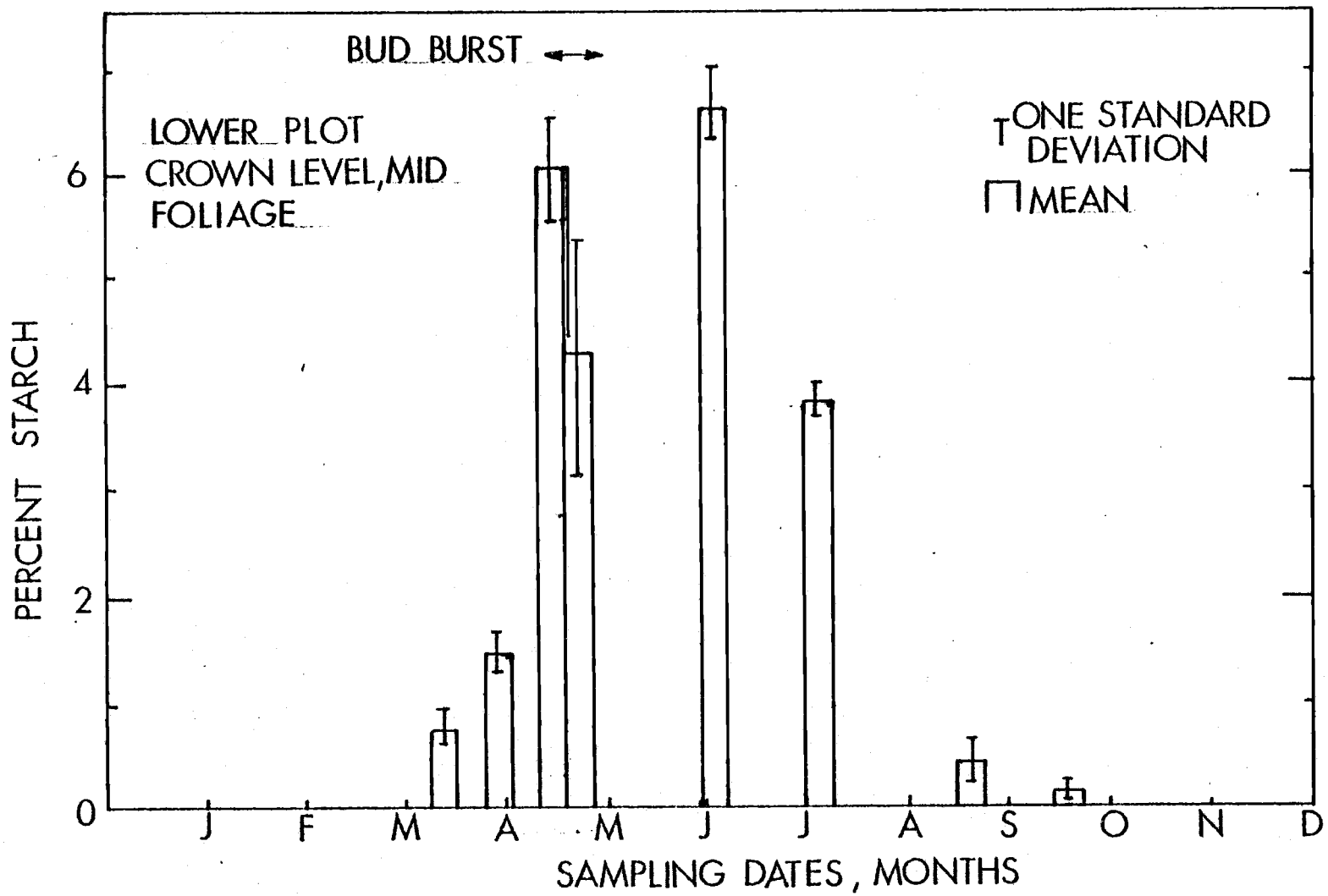
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CROWN LEVEL, MID
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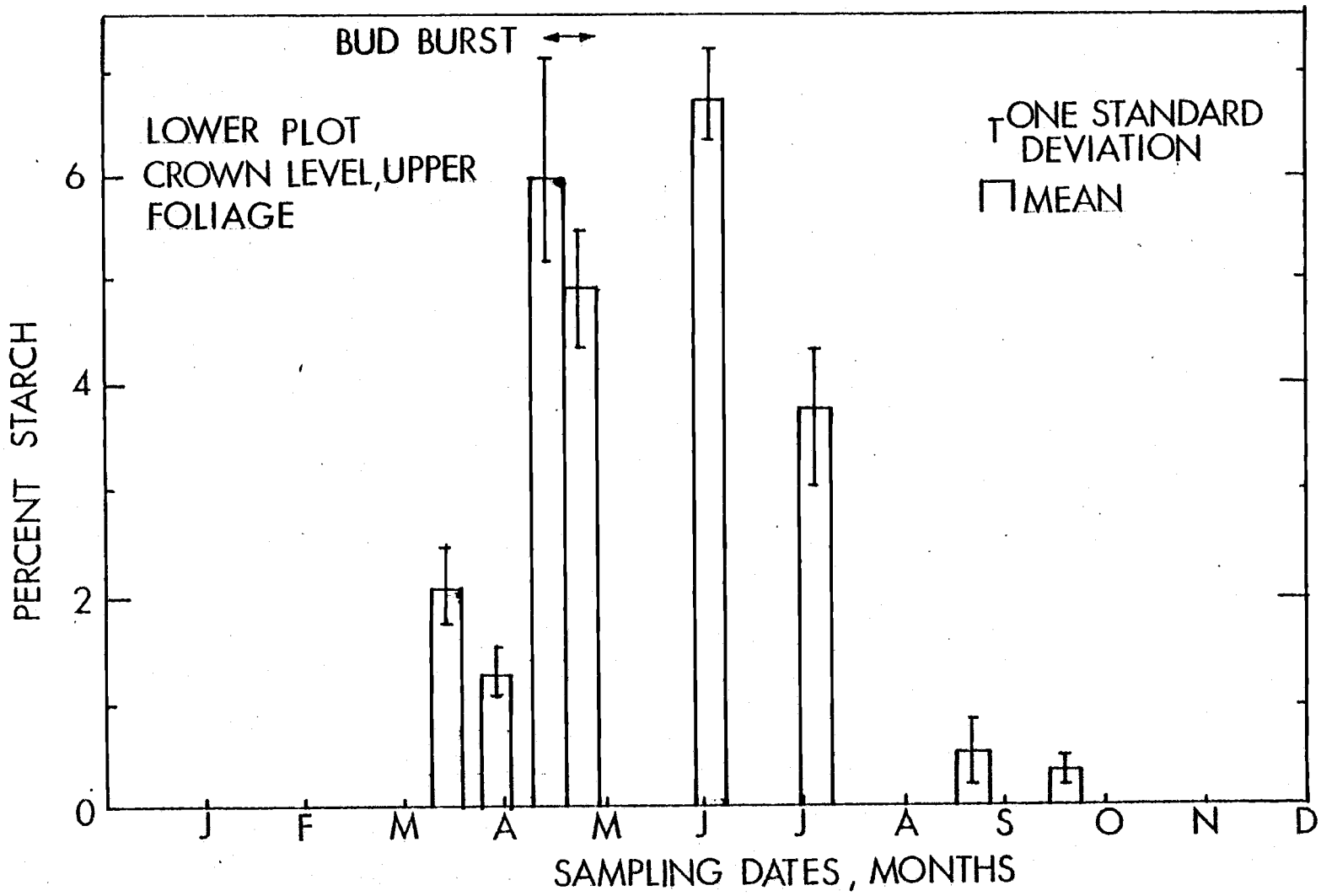
APPENDIX D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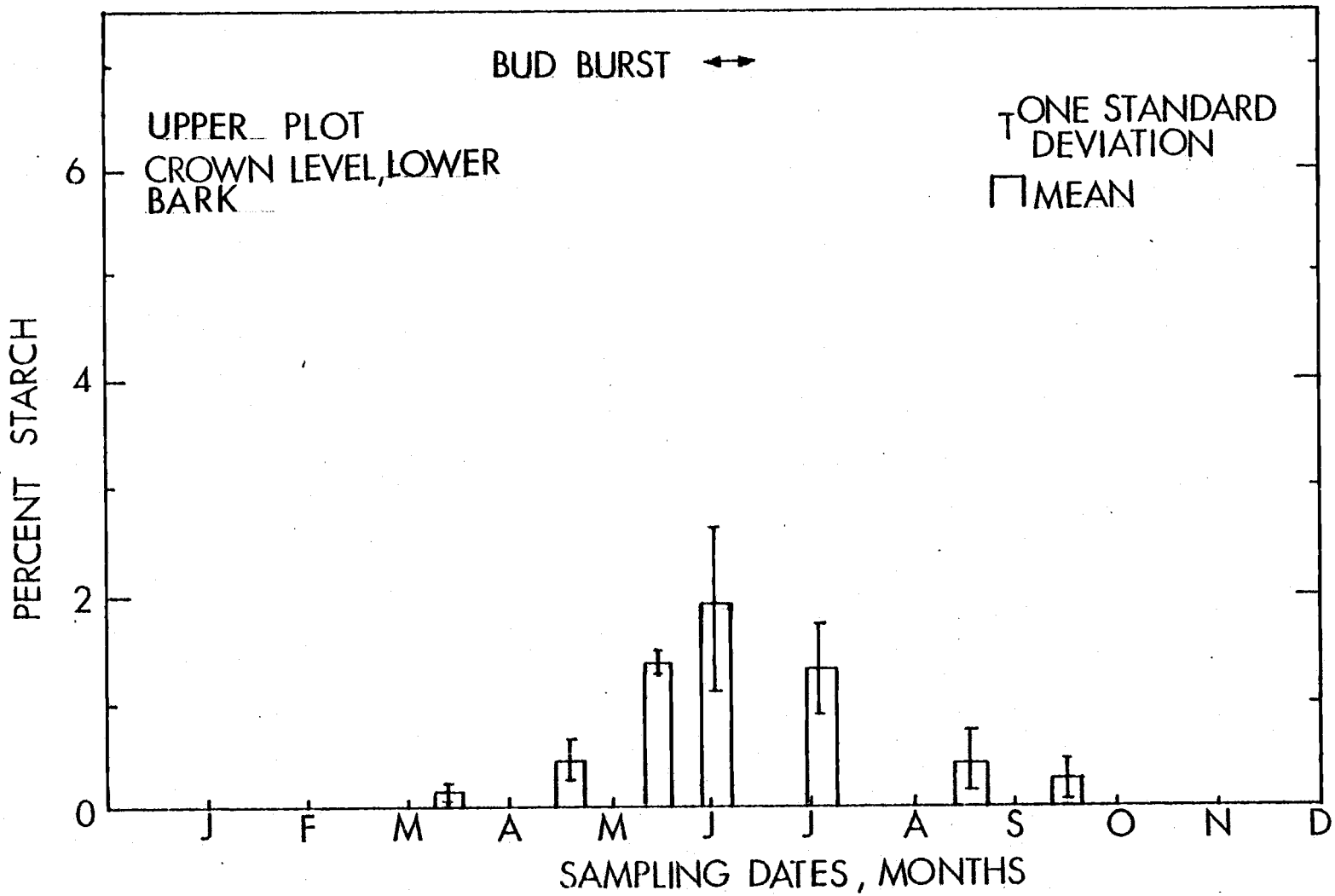
APPENDIX D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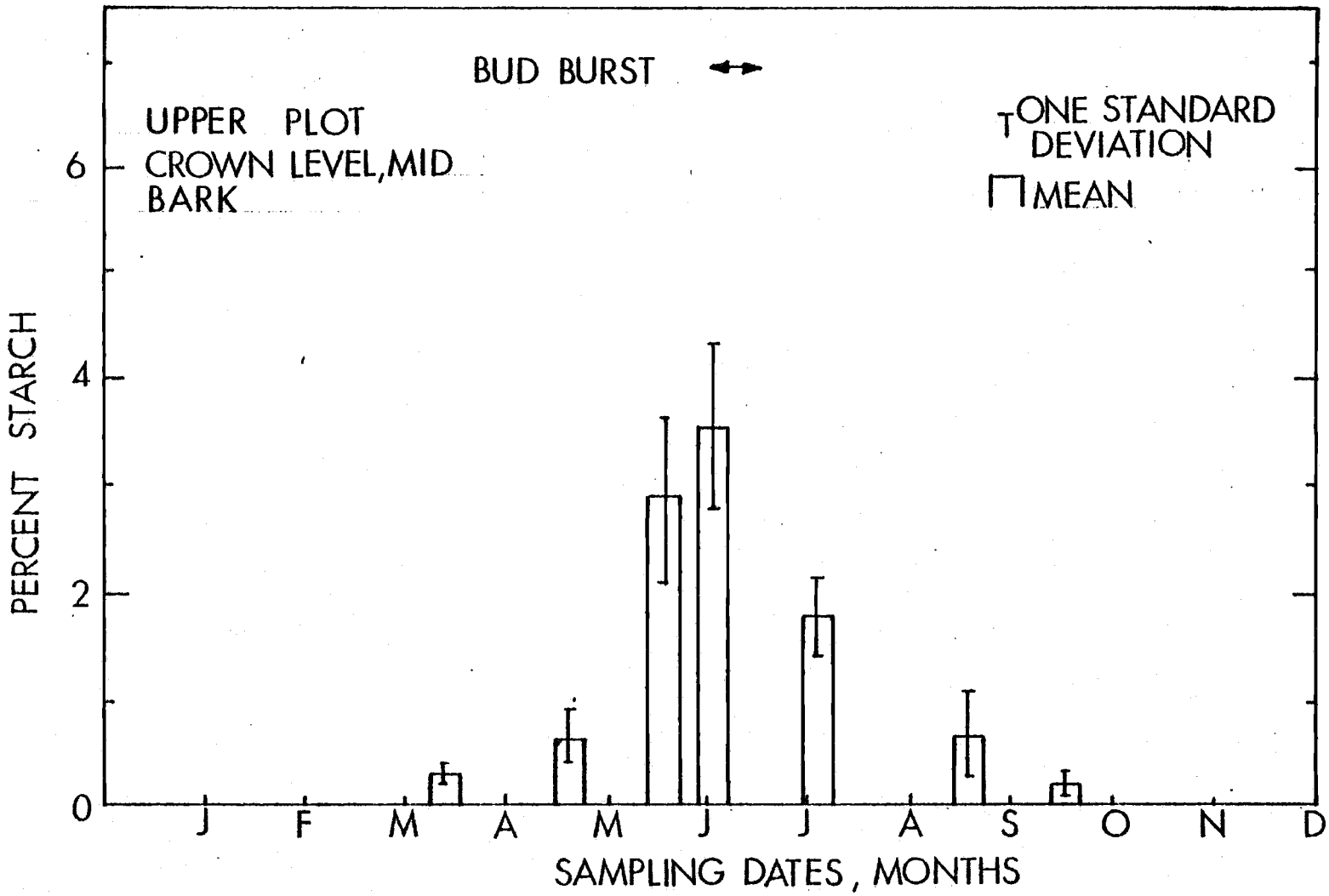
APPENDIX D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UPPER PLOT
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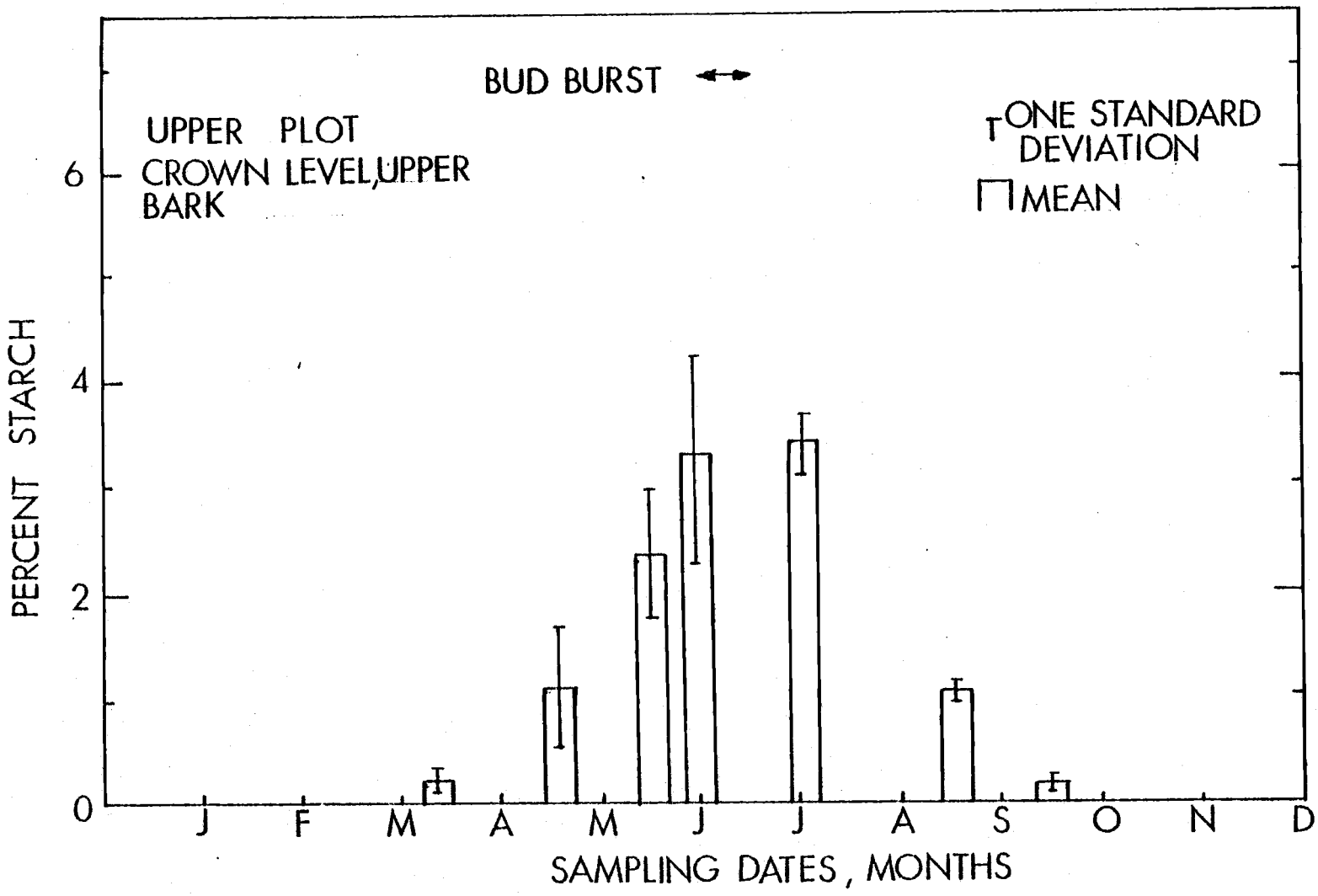
APPENDIX D


BUD BURST
UPPER PLOT
CROWN LEVEL, UPPER BARK
ONE STANDARD DEVIATION
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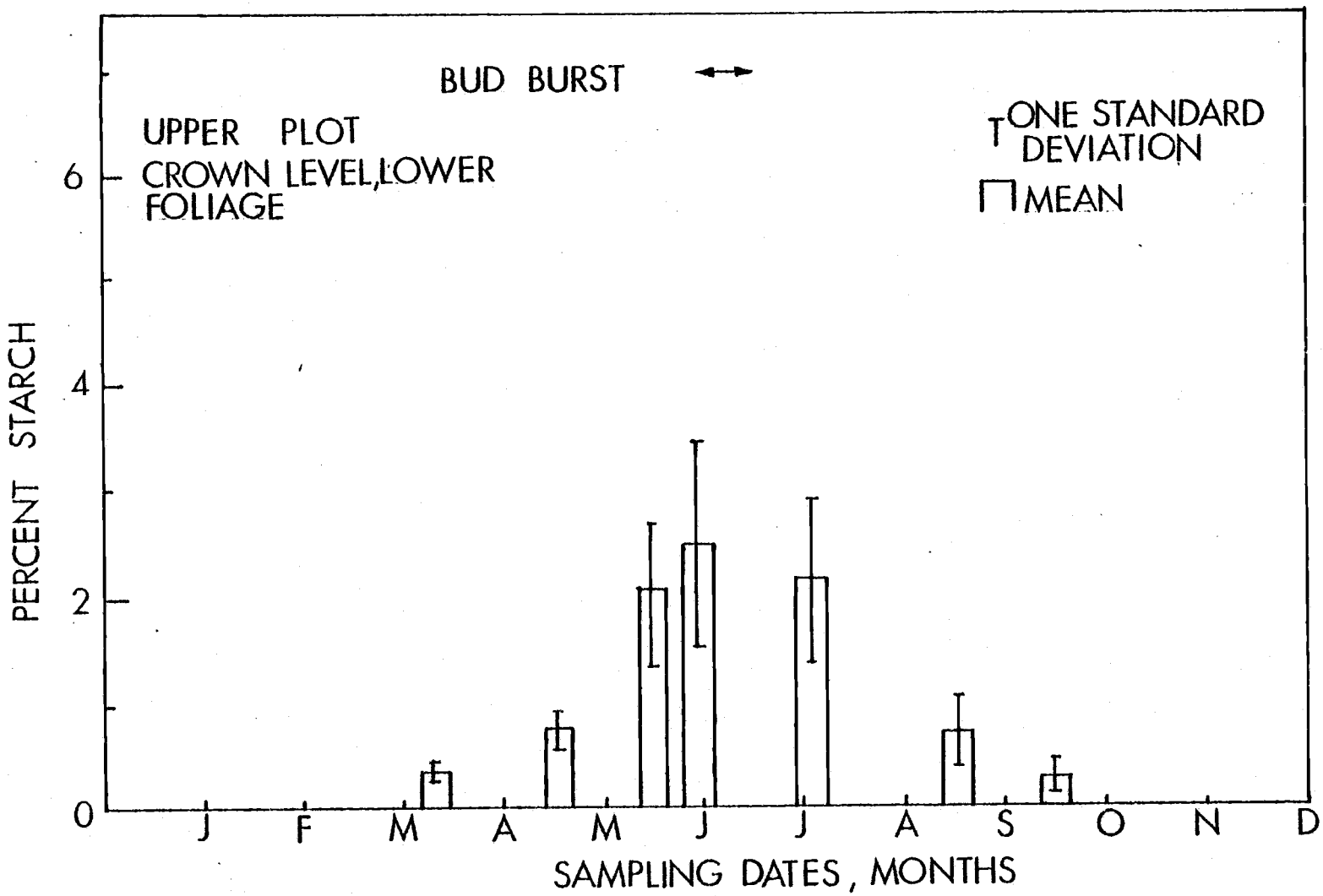
SAMPLING DATES, MONTHS

APPENDIX D


BUD BURST
UPPER PLOT
CROWN LEVEL, LOWER FOLIAGE
ONE STANDARD DEVIATION
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PERCENT STARCH
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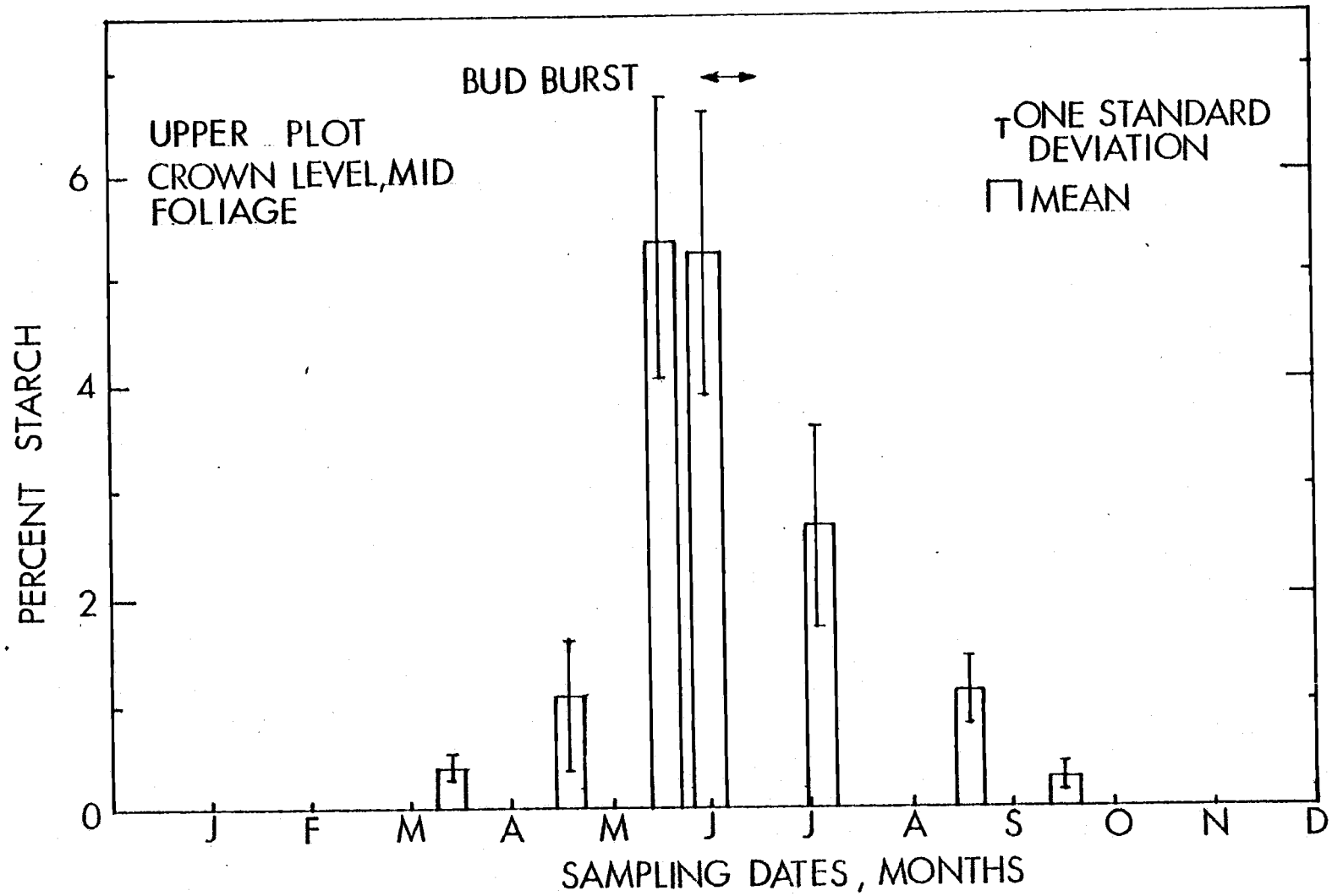
SAMPLING DATES, MONTHS

APPENDIX D


BUD BURST
UPPER PLOT
CROWN LEVEL, MID
FOLIAGE
ONE STANDARD DEVIATION
MEAN
PERCENT STARCH
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SAMPLING DATES, MONTHS

APPENDIX E

Fixing Procedure

1. Take fresh plant matter and dice into 1mm curbes. Use a sharp blade to reduce physical tissue damage.
2. Add glutaraldehyde fixative.
   3% glutaraldehyde in 0.1M PO_4 with 0.25M sucrose and 2mM $CaCl_2$
3. Fix for two hours in the cold.
4. Remove fixative.
5. Wash in 0.1M PO_4 buffer for 8 hours, changing periodically.
6. Fix in 2% OSO_4, equal volumes Osmium and 0.2M PO_4.
7. Fix in the cold and dark for two hours.
8. Wash in 0.1M PO_4 for 2 hours.
9. Dehydrate EtOH, 10-15 min. in each solution.
   25%
   50%
   75% (come to room temperature)
   95% 2 times
   100% 3 times
   Propylene oxide 3 times (P.O.)
   1 part P.O. to 1 part Spurr (hard) 3 Hours
   1 part P.O. to 2 parts Spurr 8 hours or overnight.
   Spurr alone 3 hours.
10. Capsule
11. Oven at 70^{o} for 8 hours.

APPENDIX F

Summary of absolute starch values recorded for one diel cycle

Time	Crown Level	% Starch Foliage	% Starch Bark	Time	Crown Level	% Starch Foliage	% Starch Bark
8:30 AM	Upper	7.10	3.24	8:30 PM	Upper	4.30	2.23
	Mid	7.01	3.31		Mid	6.63	3.29
	Lower	6.03	2.35		Lower	4.70	2.68
10:30 AM	Upper	3.88	2.69	10:30 PM	Upper	3.63	2.51
	Mid	7.02	3.08		Mid	5.68	3.81
	Lower	6.35	2.28		Lower	4.69	1.97
12:30 PM	Upper	3.62	2.22	12:30 AM	Upper	5.47	3.52
	Mid	7.44	3.46		Mid	5.83	2.82
	Lower	6.86	4.28		Lower	5.20	3.67
2:30 PM	Upper	4.82	3.38	2:30 AM	Upper	5.13	3.22
	Mid	5.80	3.04		Mid	7.33	3.43
	Lower	5.94	3.34		Lower	6.84	3.60
4:30 PM	Upper	5.10	3.95	4:30 AM	Upper	4.83	2.46
	Mid	6.70	3.67		Mid	4.05	1.39
	Lower	5.66	2.94		Lower	3.84	1.09
6:30 PM	Upper	6.24	3.51	6:30 AM	Upper	4.48	2.11
	Mid	5.12	2.91		Mid	4.18	1.60
	Lower	7.04	3.21		Lower	6.48	2.17

APPENDIX G

Stains

Uranium staining

9.2 grams Uranyl acetate in a 50 ml. volumetric flask, fill to 50 ml.

Lead staining

Must be prepared fresh daily

Prepare fresh 10N NaOH by adding 5.0ml. distilled deionized H_2O to 2.0 grams low caronate NaOH pellets mix well

Weigh out 20.0 mgm. lead citrate, add 10.0ml. distilled deionized (boiled)

H_2O shake well to dissolve. Add two drops 10N NaOH (fresh) cover, mox well for about 5 minues. Use only if solution completely clears.

Procedure

1. Make a 50% EtOH Uranyl Acetate solution in a small tube (spin Uranyl to remove any particulate matter). Make one drop on wax for each grid. Immerse grid cover and stain in the dark for 15 minutes.
2. Remove grid and wash in water for 30 seconds dry on edge of filter paper.
3. For Lead Citrate wet bottom of petri dish with 0.02M NaOH (removes CO_3)
   Rinse in same or water.

APPENDIX H

Fixing Procedure

1. Take fresh plant matter and dice into 1mm cubes. Use a sharp blade to reduce physical tissue damage.
2. Add glutaraldehyde fixative.
   3% glutaraldehyde in 0.1M PO_4 with 0.25M sucrose and 2mM $CaCl_2$.
3. Fix for two hours in the cold.
4. Remove fixative.
5. Wash in 0.1M PO_4 buffer for 8 hours, changing periodically.
6. Fix in 2% OSO_4, equal volumes Osmium and 0.2M PO_4.
7. Fix in the cold and dark for two hours.
8. Wash in 0.1M PO_4 for 2 hours.

9. Dehydrate EtOH, 10-15 minutes in each solution.
   25%
   75% (come to room temperature)
   95% 2 times
   100% 3 times
   Propylene oxide 3 times (P.O.)
   1 part P.O. to 1 part Spurr (hard) 3 Hours
   1 part P.O. to 2 parts Spurr 8 hours or overnight.
   Spurr alone 3 hours.

10. Capsule

11. Oven at $70^{o}C$ for 8 hours.