Nutrient Metabolism of Todomatsu
(*Abies sachalinensis* MAST.)
Seedling IV.
Variation in Carbohydrate Content of Todomatsu Seedling in Dormant Season

By

Chozaburo TAMARI

トドマツ苗木の栄養代謝 IV
休眠期における炭水化物の変動

玉利長三郎

CONTENTS

Introduction 21
Results 22
Discussion 24
Summary 27
References 28
要 約 30

Introduction

In order to ascertain why the Todomatsu seedling with lammas shoots is easily damaged by abnormal climate, as compared with the normal seedling, the author previously investigated the variation in moisture content and reducing sugar concentration in its shoot from late September to the season of snow laying. And he reported that moisture content decreased, while reducing sugar concentration increased gradually in both shoots with advancing hardiness. The moisture content of the lammas shoots was always higher than that of ordinary shoots and, on the other hand, reducing sugar concentration of the former was always lower than that of the latter, and the differences in both shoots became larger from mid-October to early November. Thereafter, it showed a tendency to lessen, but remained constant in the time when the seedlings were covered with snow.

C. TAMARI, Silviculture Laboratory, Faculty of Agriculture, Hokkaido University.

Recently, besides sugar, water-soluble protein, polyhydric alcohols, anthocyanins, and sulfhydryls et al. have been adopted as the materials which increase with the advancing hardiness. In this paper, he clarified the variations in the carbohydrate concentrations (reducing sugars, total sugars, and starch) in comparison between the normal seedling and the seedling with lammas shoots in dormant season (from late September, 1961 to early February, 1962).

Material was treated with as previously described in detail.

OKAZAKI's improved micro-Bertrand method was applied for not only reducing sugars but also total sugars and starch, and their values were converted to glucose and expressed in the ratios on the base of the values that the residue weight was taken away from a dry or a fresh weight.

Results

The results obtained in this experiment are shown graphically in Figures 1, 2 and 3.

As reported previously, reducing sugar concentration in lammas shoot (from late September to the time of snow laying) was lower than that in ordinary shoot,

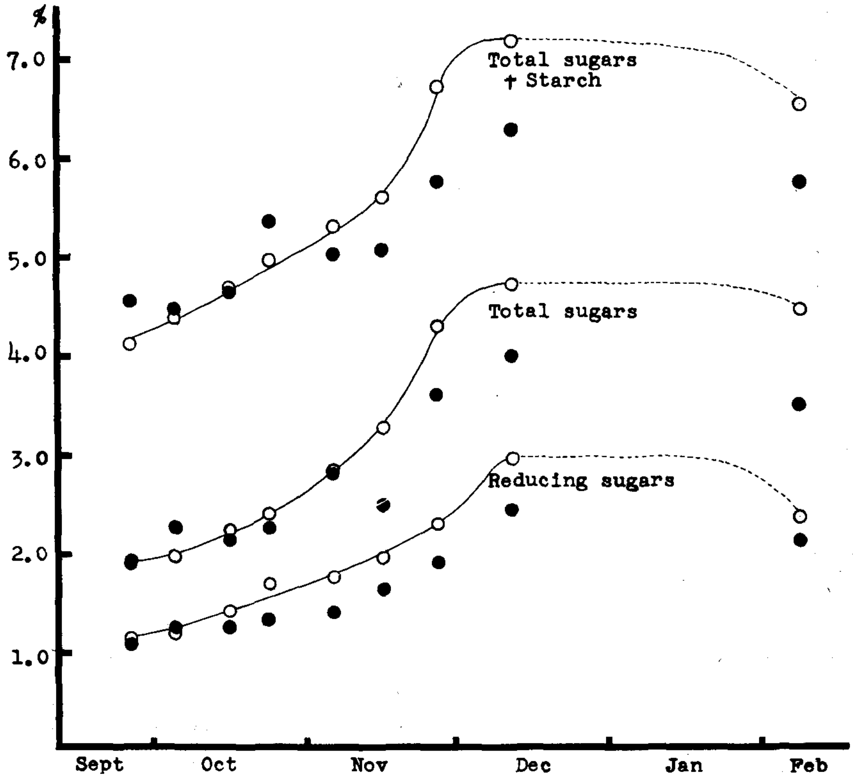

Fig 1. Variation in carbohydrate content of lammas shoot (●) and of ordinary shoot (○) in Todomatsu (*Abie sachalinensis* MAST.) seedling on a dry weight basis.

and it kept increasing throughout the abovementioned period in both shoots. Thereafter, according to the measured results during snow laying, reducing sugar concentration went up remarkably from late November to mid-December, and then showed decreasing tendency in early February, when the top of the seedling was covered over by snow. Therefore, reducing sugar concentration of Todomatsu seedling under favorable snows generally reaches to the maximum level in about mid-December, presumably constant thereafter to early February when the

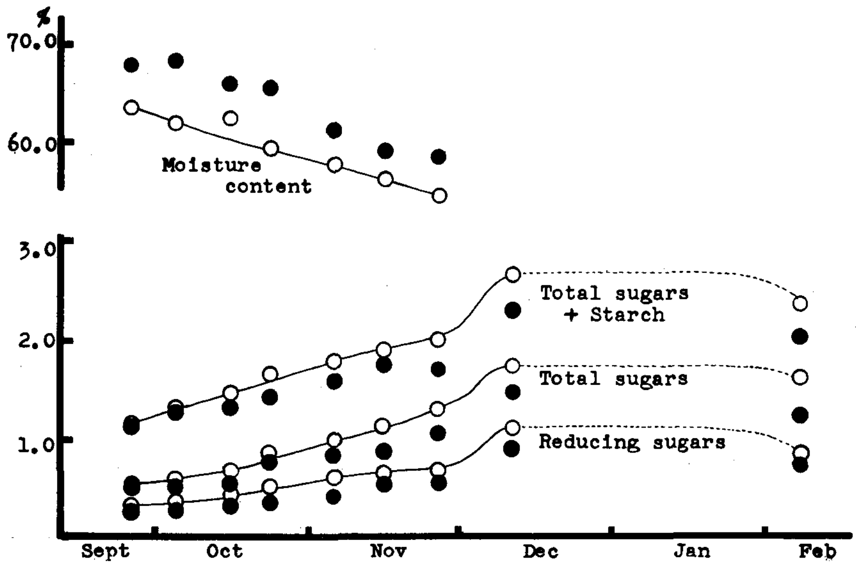

Fig 2. Variation in moisture content and carbohydrate content of lammas shoot (●) and of ordinary shoot (○) in Todomatsu (*Abies sachalinensis* MAST.) seedling on a wet weight basis.

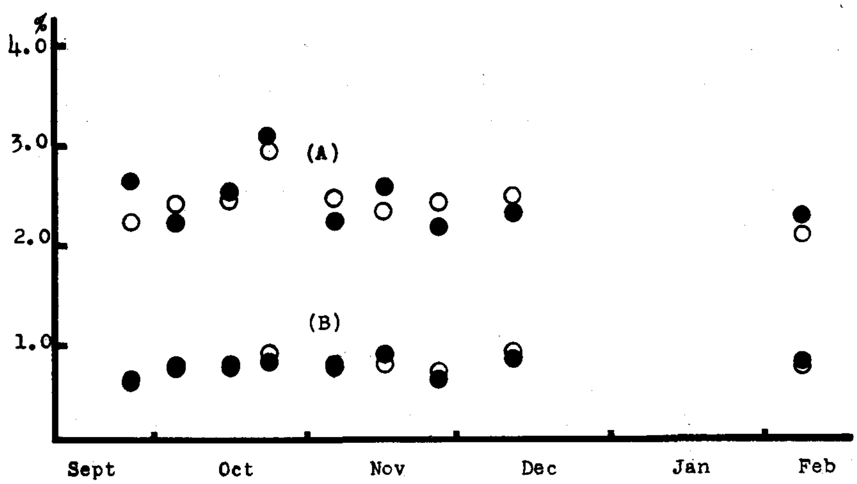

Fig 3. Variation in starch content of lammas shoot (●) and of ordinary shoot (○) in Todomatsu (*Abies sachalinensis* MAST.) on a dry (A) and a wet weight basis (B).

root begins to act in the ground. In any way, reducing sugar concentration of the lammas shoot was lower than that of ordinary shoot in dormant season and the difference between both shoots developed from late September to mid-November and thereafter became somewhat less and remained constant to early spring.

Seasonal variation of total sugar concentration from autumn to winter resembled that of reducing sugar; precisely, it began to increase in late September and increased remarkably from late November to mid-December and reached to maximum level concentration in the latter period. The increasing trend was more remarkable in total sugar concentration than in reducing sugar concentration alone, that is, the value deducted reducing sugar weight from total sugar weight increased particularly. In the total sugar concentration on a fresh weight basis, lammas shoot was lower than ordinary shoot throughout the experimental season as the results of reducing sugar. But, on a dry weight basis, there were no obvious differences between both shoots till mid-October, and the fluctuation was sharp in lammas shoot. The differences started growing in early October and reached to the maximum level in mid-December, and the maximum difference was much larger than that of reducing sugar. The degradation of total sugar concentration in early February in lammas shoot was much higher than that in ordinary shoot. On the contrary, the degradation in early February in reducing sugar concentrarion was much larger in the latter than in the former.

Starch content of ordinary shoot traced an increasing curve till late October and dropped from late October to late November in spite of the increment of sugars and then increased to the maximum level of late October in mid-December. In early February, starch value dropped somewhat below that in mid-December but this starch value was not the minimum one. The variation range of starch content was less than reducing and total sugar concentrations, and the range was limited within 0.3% on a fresh weight basis (0.9% on a dry weight basis). And also the variation of starch content made more frequent flashes than the other two and this flexibility was more remarkable in lammas shoot than in ordinary shoot. These violent changes of lammas shoot as compared with ordinary shoots were shown not only in starch content but also in moisture content and total sugar concentration.

Discussion

The amount of carbohydrates (and moisture), of course, varies with parts, ages, and growing stages of trees, hence the seasonal variation of the carbohydrate concentration differs in accord to the parts of a tree used for the experimental material, too. For example, HEPTING (1945) reported that, in April, the amount of the reserve carbohydrate of Shortleaf pine (*Pinus echinata* MILL.), including sugars, starch, and their intermediate products, was 16.6% in the needles, 12.4% in the stem bark and 2.4% in the stem wood on a dry weight basis. And also, the soluble carbohydrates after hydrolysis by 3% hydrochloric acid in TAGUCHI's study (1939) used Kuwa (*Morus bombysis* KOIDZ.) graft-seedling, were 190.6 in the bark and 129.2 in the wood of shoot, 250.8 in the bark and 152.3 in the stem wood of the root stock, 328.9 in the root-bark and 185.9 mg/cm³ in the root-wood. In this experiment, he determined simultaneously reducing sugars, total sugars, and starch of one year's lateral shoot containning needles and buds. He could not use the terminal shoot as the material, because, in most cases, the seedling with lammas shoots has no terminal shoot.

The lammas shoot had a fluctuated curve in comparison with the smooth curve in ordinary shoot and this was more remarkable on a dry weight basis than on a fresh weight basis. It seems that this is due to the deviation of the material collected and to the sensibility of lammas shoot to weather condition. As the time of the budding and the cessation of growth which the seedling with lammas shoots has differs from each other and, accordingly, each lammas shoot has the different quantity of wood part, he used five lammas shoots at random at each sampling for keeping out of this deviation, but could not perfectly exclude this deviation.

In the preceding HEPTING's paper, the carbohydrate fluctuations were limited within about 2% in stem wood and 7% in stem bark on a dry weight basis. His experiment in Todomatsu showed that the fluctuations of carbohydrates were within 3.1% in total carbohydrates, 2.8% in total sugars, 1.8% in reducing sugars, and 0.9% in starch on a dry weight basis. On a fresh weight basis, each fluctuation was within 1.5, 1.2, 0.8, and 0.3%. In PARKER's study (1958) in the bark of *Pinus strobus*, sugar concentration increased about 1.5% in the period from August to December, and this increment value was about in agreement with that of Todomatsu.

Total and reducing sugar in Todomatsu had the increasing trend from late September to mid-December and, particularly, increased markedly from late November to mid-December and it was difficult to recognize the peaks of the starch fluctuation in both shoots at that season on a fresh weight basis. But, on a dry weight basis, the starch fluctuation reached the peak in late October and decreased in the period of the sugar increase and thereafter, in mid-December, increased again somewhat. It is well-known that sugar, especially sucrose, increment from autumn to winter is owing to the starch hydrolysis; hence at that season starch decreases while sugar increases. For example, WORLEY (1937) reported that the relative amount of starch decreased with a decrease in temperature and increased again with a return to the higher temperature for *Pinus ponderosa* and *Pseudotsuga taxifolia*. In this experiment, the fluctuations in total and reducing sugars were much larger than that in starch, but the degradation of starch was much smaller in quantity as compared with the sugar increasing ratio, and thereafter starch content increased again to the maximum level. GIBBS (1940) claimed that the winter increase in sucrose does not account for all the missing starch and WILCOX (1958) suggested that this fraction is used in phloem differentiation. It seems that, if the products by photosynthesis in winter is equal to the expense for respiration, the decrease of starch may mostly correspond to the increase of sugars and that the relation between sugar and starch in the living tree is liable to vary with the weather condition, and he could not confirm clearly any preceding report by these results.

According to OKAZAKI (1952), sugar concentration in one year's leaves of Todomatsu seedling increased more remarkably in winter than in summer and especially mono-saccharide concentration was much higher in winter as compared with Ezomatsu (*Picea jezoensis* CARR.). On the other hand, SAKAI (1960) established three fundamental types; viz. reducing sugar, sucrose, and intermediate type according to the ratio of the carbohydrate constitution to the woody plants in winter and he found that Todomatsu belongs to sucrose type. In SAKAI's experiment, the leaves were used as the samples. In this experiment of one year's shoot, total and reducing sugars showed the similar increasing trend from autumn to winter and this increasing trend was rather remarkable in total sugars than in reducing sugars, and also the differences between both sugars grew much larger from late October. Namely, the remaining sugars (=non-reducing sugars) deducted the reducing sugars from total sugars (SAKAI recognized a large amount of this remainder as sucrose) went up much with the advancing hardiness though the amount of reducing sugars was much larger than that of remainder.

According to FABRICIUS' study (1905) on Norway spruce (*Picea excelsa*), and PRESTON and PHILLIPS' maintainance (1911) on *Populus deltoides* MARSH. and 8 other deciduous tree species, it was shown that the transformation of starch in the older stem is not so great as in the younger stem. Starch content of the new shoot in Todomatsu seedling flashed within 0.3% on a fresh weight basis. Moreover, SATOO and TAKEKOSHI (1952) reported that the starch fluctuation in the new shoot of Kunugi (*Quercus acutissima*), a deciduous species, was six or seven times as large as that of Sirakasi (*Q. myrsinaefolia*), a evergreen species, throughout a year, too. These results show that one year's shoot of the evergreen tree has a very narrow range in the variation of starch content. KRAMER and KOZLOWSKI (1960) presumed that the evergreen species was more dependent on current photosynthesis for shoot growth in the spring than on stored food, because the seasonal changes of the carbohydrate concentrations in the evergreen species generally show much smaller fluctuations than in the deciduous species. They, also, presumed that deciduous species accumulate them during the winter, because of reduced respiration and cessation of growth at that season.

Moisture content did not trace a smooth decreasing curve; that of lammas shoot was always much higher than that of ordinary shoot, and also the relation between both shoots in the some seedling was similar. The differences between both shoots showed a tendency to decrease, but they remained constant even in the time when the seedlings were covered with snow.

Reducing and total sugar concentration in lammas shoot were much smaller than in ordinary shoot and the differences between both shoots remained constant until early February.

On the frost-hardening process among varieties, there are two types ; one is that the stage when each variety becomes frost-hardy differs from each other but each has the equal maximum value in frost-hardiness, and the other is that each variety differs from each other not only in the stage when each variety becomes frost-hardy but also in the maximum frost-hardiness. According to the variations of moisture content, reducing and total sugar concentration (which were close in relation to frost-hardy) in this result, Todomatsu seedlings showed the latter type under favorable snowfall after late November. Growing season of the seedling with lammas shoots was longer than that of the normal one, and the former was much larger in moisture content and much smaller in reducing and total sugars than the latter. Namely, among individuals of Todomatsu seedling, it was presented that the later the cessation of growth comes, the lower the grade of frost-hardy and its maximum level are ; this fact suggests that the shortening of the growing period is indispensable for the production of cold-hardy individuals, and also, SAKAI (1955) stated that the developmental stage of twigs are important as an inner factor which is required for hardening by means of low temperatures according to his results for Kuwa varieties. The lammas shoot flashed more frequently in starch content than ordinary shoot and, furthermore, starch degradation of the former in early February was much larger than that of the latter. This trend indicates that lammas shoot is easier influenced actively by the weather condition than ordinary ones.

Summary

At the beginning of the preliminary clarification of physiological feature in Todomatsu seedling with lammas shoots, the variation in moisture, starch, reducing and total sugar contents of the seedling with lammas shoots as compared with the normal seedling, in dormant season (from late September, 1961 to early February, 1962) were measured. And the following results were obtained.

1. Reducing and total sugar concentrations increased gradually, and, conversely, moisture content degradated with the growing hardiness. The increase of sugar was most remarkable from late November to early December, and reached the maximum concentration in about mid-December. In early February, their concentrations became slightly lower. Non-reducing sugar concentration (in comparison with that of reducing sugar concentration) increased more remarkably in hardening process though the amount of reducing sugars was much larger than that of non-reducing sugars.

2. Moisture content of the lammas shoot was always higher than that of the ordinary shoot till the season of the snow laying. In contrat, lammas shoot till the season of the snow laying. In contrast, lammas shoot always had much

less carbohydrate concentration than ordinary shoot in the dormant season. The differences in reducing and total sugar concentrations between both shoots began to occur in mid-October and increased until early December. The time of maximum concentration was the same in both shoots but the difference ramined constant until early spring.

3. The range of the variation in starch content in the preceding period was very narrow in comparison with reducing and total sugar concentrations, and starch content reached to the maximum level in late October and thereafter has a decreasing tendency with the increment of sugar though the degradation of starch was much smaller as compared with the sugar's increment. Starch content in lammas shoot varied more frequently, and the range of its variation was larger than that of ordinary shoot.

4. These results indicated that the later the cessation of growth comes, among individuals of Todomatsu seedling, the lower the grade of the frost-hardy and its maximum level are; this fact suggest that the shortening of growing period is indispensable for the production of cold-hardy individuals, and therefore lammas shoot is damaged easily by the abnormal weather.

The author wishes to express deep appreciation to Emeritus Professor K. KONDA for his suggestions and encouragement throughout the course of this work, to Professor Y. SAITO and Assistant Professor K. MUTO for their continued guidance and cordial revision and to Assistant Professor A. SAKAI, Research Institute of Low Temperature Science, who gave him kind leading in the determination of sugar concentration.

The seedlings used in this experiment were obtained from the Sapporo Nursery of the College Experimental Forest, Hokkaido University.

References

1) FABRICIUS, L.: Untersuchungen über den Stärke- und Fettgehalt der Fichte auf den oberbayerische Hochebene. Naturw. Zeit. Land- u. Forstw., **3**, 137–176, 1905.

2) GIBBS, R. D.: Studies in tree physiology. II. Seasonal changes in the food reserves of field birch (*Betula populifolia* MARSH). Cited from 6, 1940.

3) HEPTING, G. H.: Reserve food storage in Shortleaf pine in relation to little-leaf disease. Phythopath., **35**, 106–119, 1945.

4) KONDA, K., TANIGUCHI, M., and TAMARI, C.: An example of the cold-damage of *Abies sachalinensis* MAST. seedling in the nursery. (Japanese) Trans. 66 th Meet. Jap. For. Soc., 92–93, 1956.

5) KONDA, K., MUTO, K., TANIGUCHI, M., and TAMARI, C.: Ecological observations on the cold-damage of *Abies sachalinensis* MAST. by the snow removing method. (Japanese) Trans. 9 th Meet. Hokkaido Branch Jap. For. Soc., 26–29, 1960.

6) KRAMER, P. J. and KOZLOWSKI, T. T.: Physiology of trees., 1960.

7) KOZLOWSKI, T. T. and PETERSEN, A. C.: Variation in moisture contents of dormant buds. For. Sci. **6**, No. 1, 61–66, 1960.

8) LEVITT, J., SULLIVAN, C. Y., JOHANSSON, N., and PETTIT, R. M.: Sulfhydryls—A new

factor in frost resistance I. Changes in SH content during frost hardening. Plant Physiol., **36**, 611-616, 1961.

9) OKAZAKI, A.: Some hydro-physiological properties of seedlings of Todomatsu, Ezomatsu, and Akaezomatsu. (Japanese) Rep. Coll. Exp. For. Kyoto Univ., **3**, 9-13, 1952.

10) OKAZAKI, A.: Physiology of trees., (Japanese) 1960.

11) PARKER, J.: Changes in sugars aud nitrogenous compounds of tree barks from summer to winter. Naturw., **45**, 139, 1958.

12) PARKER, J.: Seasonal variations in sugars of conifers with some observations on cold resistance. For. Sci., **5**, 56-63, 1959.

13) PARKER, J.: Seasonal changes in cold resistance of some northeastern woody evergreens. J. Forestry, **59**, 108-111, 1961.

14) PARKER, J.: Relationships among cold hardiness, water-soluble protein, anthocyanins, and free sugars in *Hedera helix* L. Plant Physiol., **37**, 809-813, 1962.

15) PRESTON, J. F. and PHILLIPS, F. J.: Seasonal variation in the food reserves of trees. For. Quartely, **9**, 232-243, 1911.

16) SAKAI, A.: The relationship between the process of development and the frost hardiness of the Mulberry tree. (Japanese) Low Temp. Sci., Ser. B, **13**, 21-31, 1955.

17) SAKAI, A.: The frost-hardening process of woody plant I. Relation of increase of carbohydrate and water soluble protein to frost-resistance in tree (1). (Japanese) Low Temp. Sci., Ser. B, **15**, 17-29, 1958.

18) SAKAI, A.: Ditto. II. Relation of increase of carbohydrate and water soluble protein to frost-resistance in tree (2). (Japanese) Ibid., **16**, 23-34, 1958.

19) SAKAI, A.: Ditto. V. The relationship between developmental stage and frost-hardening. (Japanese) Ibid., **17**, 43-49, 1959.

20) SAKAI, A.: Ditto. VI. Seasonal variation in sugars (1). Japanese) J. Jap. For. Soc., **42**, 97-102, 1960.

21) SAKAI, A.: Ditto. VII. Seasonal variation in sugars (2). (Japanese) Low Temp. Sci., Ser. B, **18**, 1-14, 1960.

22) SAKAI, A.: Ditto. VIII. Relation of polyhydric alcohols to frost hardiness. (Japanese) Ibid., **18**, 15-22, 1960.

23) SATOO, T. and TAKEKOSI, T.: Seasonal change of starch content in *Quercus myrsinaefolia* and *Q, acutissima.* (Japanese) Misc. Information, Tokyo Univ. Forests, **9**, 17-23, 1952.

24) SCHMUTZ, W., SULLIVAN, C. Y., and LEVITT. J.: Sulfhydryls—A new factor in frost resistance. II. Relation between sulfhydryls and relative resistance of 15 wheat varieties. Plant Physiol., **36**, 617-620, 1961.

25) SIMINOVITCH, D., WILSON, C. M., and BRIGGS, D. R.: Studies on the chemistry of the living bark of the Black Locust in relation to its frost hardiness. V. Seasonal translocations and variations in the carbohydrates: Starch-sucrose interconversions. Plant Physiol., **28**, 383-400, 1953.

26) SIMURA, T., WATANABE, A., and KANOO, T.: Physiological studies on the cold resistance of the tea plant. (Japanese) Proc. Crop Sci. Soc. Japan, **23**, 121, 1954.

27) TAGUCHI, R.: Untersuchungen über die jahreszeitlich Änderung des Wasser- und Reservestoffgehaltes der Stengel und Wurzeln von Maulbeerbaum. (Japanese) J. Coll. Agric. Kyushu Imp. Univ., **8**, 350-373, 1939.

28) TAMARI, C.: Variation in moisture content and reducing sugar concentration in hardening process of Todomatsu (*Abies sachalinensis* MAST.) seedling with lammas shoot. Hokkaido univ. For. Bull., **21**, 409-414, 1962.

29) WENGER, K. F.: The sprouting of Sweetgum in relation to season of cutting and carbohydrate content. Plant Physiol., **28**, 35–49, 1953.
30) WILCOX, H.: Personal communication. Cited from 6, 1958.
31) WORLEY, C. L.: Carbohydrate changes within the needles of *Pinus ponderosa* and *Pseudotsuga taxifolia*. Plant Physiol., **12**, 755–770, 1937.

要　　約

秋から冬にかけての芽条の硬化過程において，生長終期のおくれた2次生長苗が，正常苗にくらべて含水量と還元糖含量でどういう相違をしめすかを先報で報告した。ここでは，全糖および澱粉の含量をも含めて，休眠期の炭水化物の一般的な変化をとらえ，その中で2次生長苗の生理的特性を検討した。資料，分析方法ともに先報に準じておこなった。

トドマツ苗木の還元糖，全糖濃度はすでに9月末には増大傾向にあり，11月下旬から12月初旬の間に著しい増加をしめし，12月中旬に最大含量に達した。2月初旬には，いくらか濃度は低くなっている。糖の増大期では還元糖より非還元糖の増大が多く，酒井が葉を資料として測定したと同様であった。この2つにくらべると澱粉の変動範囲は狭いが，10月下旬に最高濃度になり，その後，糖の増大にともない減少した。しかし，量的には澱粉の減少と糖の増加は一致しなかったし，また12月中旬には再び澱粉濃度は増加した。

2次生長部は正常部にくらべて含水量は多く，還元糖，全糖はともに低濃度で越冬するし，また，澱粉の変動幅が大きく，変動もはげしいことが特徴をなしていた。これはトドマツの個体間でも，生長終期のおそい苗木ほど硬化がおくれ，硬化度の低いままで越冬するので，積雪の保護をうけない秋末とか春先，あるいはまた，積雪が異常にすくない年は休眠期間中でも，諸害に対する抵抗性の弱いことをしめしている。12月頃とくらべて，2月初旬の澱粉の減少量が2次生長部でいちじるしいことも，2次生長苗が外囲環境の影響をうけやすいことをしめしていた。