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슬래쉬소나무의 花芽原基 形成의 生理學的 硏究 (Ⅱ)

頂芽의 炭水化物과 窒素 新陣代謝와 雌花 原基形成과의 關係[*1]

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Physiology of Strobilus Initiation in Slash Pine II. Ovulate Strobilus Initiation in Relation to Carbohydrate and Nitrogen Metabolism of Terminal Buds.[*1]

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슬래쉬 소나무의 花芽原基 形成 期間 中에 頂芽의 營養 狀態를 究明하기 위하여, 頂芽의 炭水化物과 아미노산의 함량을 調査하였다.

接木으로 조성된, 18年生 슬래쉬 소나무를 開花量의 多少에 따라 多數開花 그룹과 少數開花그룹으로 나누고, 각 그룹에서 각각 二個씩의 頂芽型을 선택하였다. 즉 ① 多數開花그룹에서 樹冠上部에 있는 大芽(雌花生産)와 下部에 있는 小芽(雄花生産), ② 少數 開花 그룹에서 樹冠上部에 있는 大芽(營養芽)와 下部에 있는 小芽(雄花生産)의 도합 4가지였다. 7月 하순부터 9月 상순 사이에, 頂芽를 4回 채취하여 75% 에칠알콜로 추출하고, 炭水化物은 가스크로마토그래피로, 아미노산은 自動分析器로 分析하였다.

두 그룹의 大芽는 각각 같은 그룹의 小芽보다 糖의 含量이 많았으며, 4그룹에서 共히 fructose와 glucose가 主成分을 이루고 있었다. 아미노산 中에서는 arginine의 含量이 가장 많았으며, 4그룹의 平均値로 볼 때 7月에 23%에서 9月에 60%로 增加하였다. 雌花를 生産하는 頂芽의 arginine과 總 아미노산의 함량은 다른 세그룹의 含量보다 훨씬 적었다. 多數開花 그룹내의 두 頂芽型을 비교할 때, 雌花 눈의 arginine量은 7月에 雄花 눈과 거의 같았으나, 9月에는 雌花눈이 雄花눈보다 四倍의 arginine을 含有하고 있었다. 이로 미루어 보아, arginine은 雌花의 原基形成에 직접 관여하지 않는 것으로 사료된다.

雌花를 生産하는 頂芽는 낮은 아미노산의 含量으로 因하여, 炭水化物과 아미노산의 比率(C/N率)이 原基形成 期間 동안에 아주 높은 것으로 나타났으며, 이는 雌花의 原基形成은 一時的인 新陣代謝 活動의 減少를 同伴하는 것 같다.

Soluble carbohydrates and free amino acids in the terminal buds of *Pinus elliottii* were analyzed to understand the nutritional status of the buds during the period of female strobilus initiation.

Grafted, 18-year-old slash pine trees in a seed orchard were divided into two groups, abundant-flowering (AFG) and poor-flowering group (PFG) according to their flowering history. Four types of terminal buds, with two types from each group, were examined: (1) large buds in upper crown (female-producing buds) and small buds in lower crown (male-producing) in AFG, (2) large buds in upper crown (vegetative buds) and small buds in lower crown (male-producing) in PFG. Bud samples were collected four times

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from late July to early September. Free sugars and free amino acids (75% ethanol-soluble) were determined by gas chromatography and automatic analysis, respectively.

Sugar content in the large buds of both groups was greater than in the small buds of the same group. Fructose and glucose were major sugars found in the bud tissue. Arginine was the most abundant amino acid in all four types of buds, with the concentration increased from 23% in late July to 60% in early September. Arginine and total amino acid content in the female-producing buds of AFG was much lower than three other types of buds. When female-producing buds and male-producing buds of AFG were compared in their arginine content, the former contained about same amount as the latter in late July, but showed one-fourth of the latter in early September. The low level of argining in the female-producing buds suggested a minimal or negative role of arginine in the initiation of female flower primordia.

A higher sugar to amino acid ratio was observed with female-producing buds of AFG than with vegetative or male-producing buds of either flowering group. The low amino acid content in the female-producing buds suggested that initiation of female strobilus primordia was associated with temporary reduction in the metabolic activity of the buds.

INTRODUCTION

In conifers there is a long-standing evidence that supports a positive role of light in cone production. The amount of light received by individual trees seems to be related to their capacity to produce cones (Fowells and Schubert, 1956). Cultural treatments which increase light interception by individual trees, such as thinning and branch pruning have been proposed to promote flowering through enhancement of photosynthesis and subsequent accumulation of carbohydrates. Kramer and Kozlowski (1960) stated that a high level of carbohydrates was required for cone production in woody plants. More specifically, a high carbohydrate to nitrogen ratio (C/N) has been proposed to promote flowering (Kraus, 1925).

Recently fertilization with nitrogen has been employed to stimulate flowering in many pine seed orchards. Particularly, there have been some reports indicating that stimulation of female flowering was associated with increased arginine content in the tissue after the fertilization (Barnes and Bengtson, 1968; Sweet and Hong, 1978). The stimulating effect of nitrogen fertilization appears to be one of the experimental observations contradicting the C/N theory. However, it is uncertain if nitrogen is a limiting factor in flower initiation. There have been some reports of stimulatory effect of nitrogen deficiency on flower initiation (Kuo, 1973; Lyr and Hoffmann, 1964), indicating the indirect role of nitrogen in flower initiation.

The objective of the present study was to determine the physiological conditions of terminal buds at the time of floral bud initiation in relation to the levels of alcohol-soluble carbohydrates and amino acids.

MATERIALS AND METHODS

The slash pine (*Pinus elliottii* var. *elliottii* Engelm) seed orchard used in this study was established in 1956 with grafted seedlings, and was located in Gainesville, Florida in U.S.A. Classification of trees into abundant- and poor-flowering groups and classification of terminal buds into four types were described in the first paper of this series (Lee, 1980). In the abundant-flowering group (AFG), two types of terminal buds were recognized: 1) large buds in the upper part of crown which had produced female flowers in previous years and were expected to produce female flowers the following year. 2) small buds in the lower part of crown which were

expected to produce male flowers. In the poor-flowering group (PFG), two types of terminal buds were also recognized: 1) large buds in the upper part of crown which had produced no female flowers. 2) small buds in the lower part of crown which were expected to produce male flowers.

The terminal buds were collected on July 28, August 4, August 26, and September 2. The buds were cut and frozen in liquid nitrogen in the field. The frozen buds were brought to the laboratory and ground with a mortar and pestle to fine powder. The ground bud tissue was freeze-dried and stored in a freezer for further analyses.

Extraction of Soluble Carbohydrates and Amino Acids :

About 0.5 g of freeze-dried bud tissue was extracted with 25 ml of 75% ethanol for 20 min. at 70°C and filtered. The residue was extracted again with 25 ml of fresh 75% ethanol at 70°C for 1 hour and the extract was filtrated. The extraction was repeated once more using the same amount of fresh ethanol. The extracts were combined in a flask and concentrated to about 15 ml with a rotary evaporator at 35°C to remove alcohol. The concentrate was trnsferred to a separatory funnel, and pH of the solution was adjusted to 3.0 with 0.1 N HCl. Twenty ml of hexane was added to the concentrate and partitioned into an aqueous and a hexane fraction. The hexane fraction was discarded and the aqueous fraction was partitioned with hexane two more times. The resulting aqueous fraction was centrifuged for 20 min. at 27,000 g. The supernatant was subjected to cation exchange chromatography.

Ion Exchange Chromatography :

For cation exchange chromatography Dowex 50W-X8 resin (hydrogen form, 200-400 mesh) was used as described by Splittstoesser (1969). The resin was prepared in a 1.2 cm (internal diameter) x 15 cm long glass column, and washed first with 75 ml water. Then the bud extract (supernatant of step 'M' in Fig. 1) and 25 ml water in order were run through the column and the eluate (called an acidic fraction) was saved. Then the column was washed in order with 50 ml 0.5 N NH_4OH, 25 ml water, 25 ml 4 N NH_4OH and finally with 25 ml water. The eluate (called a basic fraction) was saved. The elution rate was kept about .4 ml per minute under slight air pressure.

For anion exchange chromatography Dowex 1-X8 (formate form, 200-400 mesh) was prepared in a glass column and washed with 75 ml water. The acidic fraction (step 'P' in Fig. 1) was brought to a final volume of 100 ml, and 50 ml aliquot was adjusted to pH 8.0 with 1 N and 0.1 N NH_4OH solution, and run through the resin column. The eluate was saved. Then the resin was washed with 25 ml water, and the eluate was combined with the previous one. This combined eluate was called a neutral fraction and contained sugars (step 'V' in Fig. 1). The resin which adsorbed organic acids was washed with 50 ml 8 N formic acid and with 25 ml water. This eluate contained organic acids (step 'W' in Fig. 1).

Gas Chromatography of Sugars :

The neutral fraction after anion exchange chromatography (step 'V' in Fig. 1) was used for gas liquid chromatography of sugars. Methods described by Ford (1974) and Fretz *et al.* (1970) were used.

a) Instrumentation: The apparatus used was Packard Becker Gas Chromatograph Model 421 equipped with dual hydrogen-flame ionization detector. Separation of sugars was performed with a Pyrex glass column (U-shape, 0.64 cm O.D. x 182 cm) packed with 3% SE-30 (G.C. grade) on Chromosorb WHP (80-100 mesh, obtained from Applied Science Laboratories,

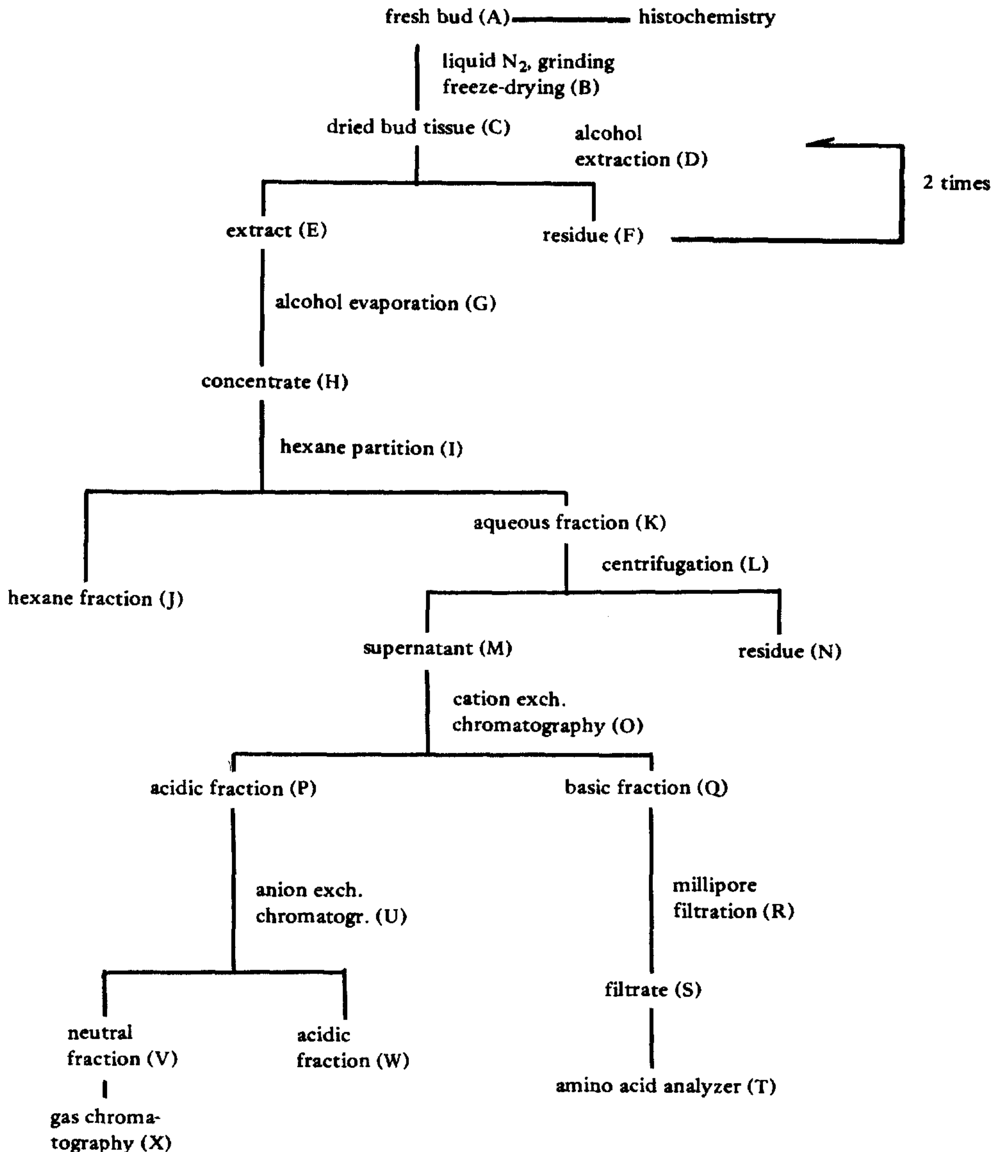


Fig. 1. Extraction procedures for free sugars and free amino acids in the bud tissue of *Pinus elliottii.*

Inc.).

b) Operating conditions: Injection port and detector temperatures were maintained at 275°C. Column temperature was initially 125°C for 5 min., programmed to rise linearly at 4°C/min. to 260°C, and held at 260°C for 10 min. Three microliter sample was injected into 'A' column, while 'B' column served for compensation of base line drift during temperature programming. Nitrogen was used as a carrier gas at a flow rate of 25 ml/min. Hydrogen and air flow rates were 25 ml/min. and 250 ml/min., respectively. A new column was conditioned at 280°C for 24 hours before use. A strip chart recorder from Honeywed (Model Electronik 196) was used at chart speed of 1.3 cm/min.

c) Preparation of sugar standards: The sugar standards used here include L-arabinose, D-xylose, D-ribose, D-glucose, D-fructose, D-mannose, D-galactose, L-rhamnose, L-fucose, and sucrose. The sugar alcohol standards were xylitol, adonitol, mannitol, D-sorbitol, and pinitol. Twenty mg of each sugar was dissolved in 20 ml anhydrous pyridine, and 0.5 ml of the sugar solution was mixed well with 0.1 ml 1-(trimethylsilyl)-imidazole (purchased from Eastman Kodak Co.) in a glass vial, and left in room temperature for 10 min. One microliter aliquot of the silylated sugar solution was injected with a microsyringe into gas chromatograph.

d) Sample preparation: The neutral fraction (step 'V' in Fig. 1) was made to a volume of 100 ml. Twenty mililiter aliquot was pipetted into a boiling flask, dried completely under vacuum, redissolved with 2.0 ml anhydrous pyridine. An 0.5 ml aliquot was pipetted into a small glass vial and mixed well with 0.1 ml 1-(trimethylsilyl)-imidazole. A stopper made of rubber should be avoided while mixing and shaking above mixture. After 10 min. 3 μl aliquot of the silylated solution was injected into the gas chromatograph. Each sample was chromatographed in triplicate.

e) Identification and quantification of peaks: Peaks in the sample chromatogram were identified by comparing retention times with those of known standard sugars. An individual sugar was quantified by using a ratio of sample peak area (area under the peak) to that of corresponding standard sugar. Total sugar content was expressed in mg/g dry weight.

Determination of Free Amino Acids :

The basic fraction (step 'Q' in Fig. 1) which contained amino acids was dried completely under vacuum, redissolved with 10 ml of 0.01 N HCl, and filtered with millipore filter (pore size 0.2 μ). The filtrate was analyzed for amino acids using an amino acid analyzer (Japan Electron Optics Laboratory Co., Model JLC-6AH). A standard amino acid solution (commercially made) containing 100 n moles/ml of each amino acid commonly found in proteins was used to quantify individual amino acids. The following formula was used for each amino acid:

$$\frac{\text{peak area (sample)}}{\text{peak area (standard)}} \times 100 \text{ n moles/ml} \times \frac{\text{sample volume (ml)}}{\text{sample weight (g)}} = \text{n moles/g.}$$

RESULTS

Gas Chromatography of Sugars :

Separation of L-arabinose, L-rhamnose, D-ribose and L-fucose was improved by maintaining the initial temperature at 125°C for 5 min., since these sugars separated poorly if initial temperature was maintained higher than 125°C or less then 5 min. at 125°C (Fig. 2). Temperature rise programmed at 4°C/min. gave a better separation of both pentoses and hexoses than programmed at 5°C/min. or higher.

Two anomer (α and β) peaks were observed in D-xylose, D-mannose, D-fructose, D-galactose, and D-glucose. It was shown that during chromatography α- and β-anomers of mannose maintained a ratio of 4:1, and those of fructose a ratio of 1:1. Thus, it was possible to calculate the contribution of mannose to the total peak when both mannose and fructose were present in the sample.

Sugar Content in the Terminal Buds :

Fig 3 shows total (Fig. 3 A) and individual sugars (Fig. 3 B-G) in the four types of terminal buds. A high amount of pinitol (15-21% of total sugars) was detected in all the samples. About half of the total sugar concentration was attributed to glucose and fructose in all four types of buds.

The total sugar concentration (Fig. 3 A, a

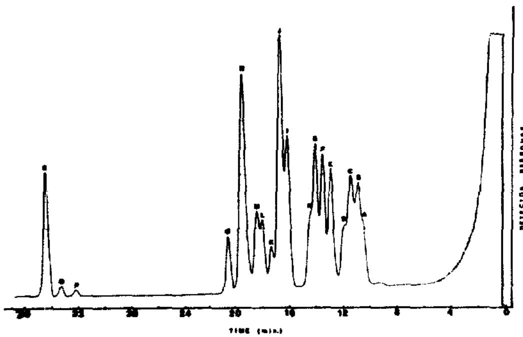
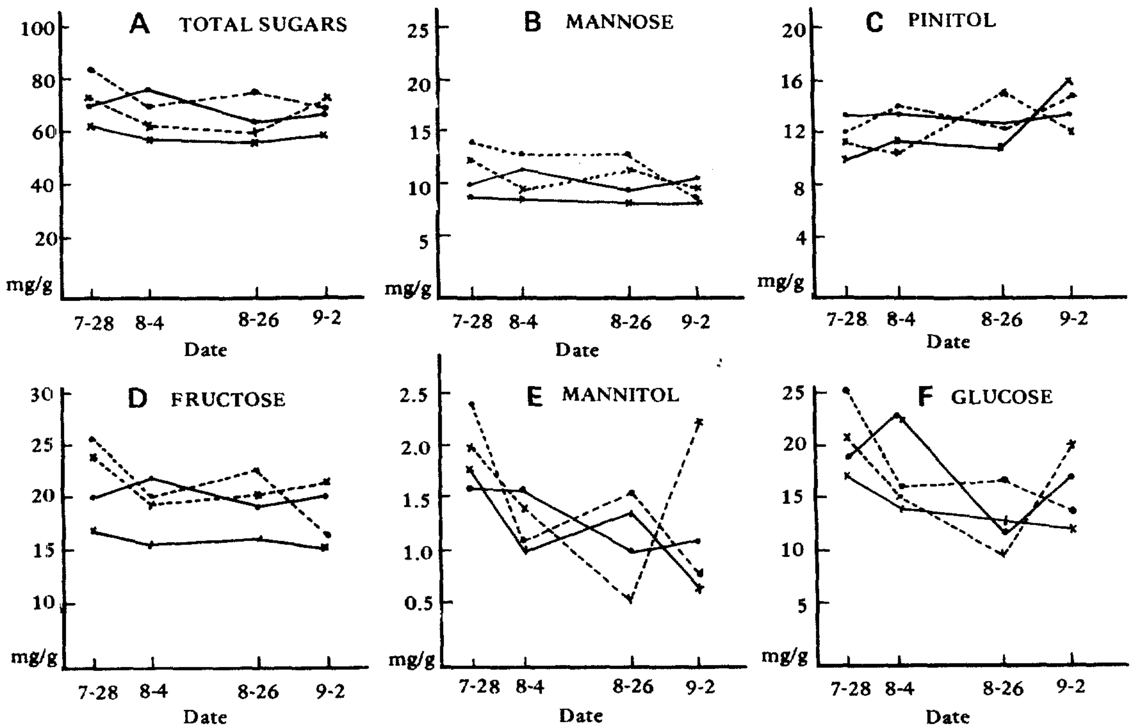


Fig. 2. Gas chromatographic analysis of trimethylsilyl derivatives of standard sugars on a 3% SE-30 column which was temperature-programmed with initial temperature of 125°C for 5 min., rising at 4°C/min. to final temperature of 260°C for 10 min.

Peak identities: A) L-arabinose, B) L-rhamnose, C) D-ribose, D) L-fucose, E) D-xylose, F) xylitol, G) adonitol, H) D-xylose, I) D-mannose and D-fructose, J) pinitol, K) D-galactose, L) α-D-glucose and D-fructose, M) D-galactose and D-mannose, N) mannitol and sorbitol, O) β-D-glucose, P, Q, and R) sucrose.

sum of individual sugars identified) remained relatively stable during the sampling period in all the four types of terminal buds. When the total sugars were compared within a flowering group, large buds of both flowering groups had a greater amount of total sugars than small buds of the same group (statistically significant at 5% level). When the total sugars were compared between the two flowering groups, no difference was observed between the large buds of AFG (female-producing buds) and those of PFG (vegetative buds).

As shown in the Fig. 3 B-G, all the individual sugars fluctuated considerably with the exception of mannose. The mannitol and glucose level (Fig. 3 E and F) decreased gradually over the sampling period. No single sugar concentration appears to be related to the female-flowering potential of the terminal buds.



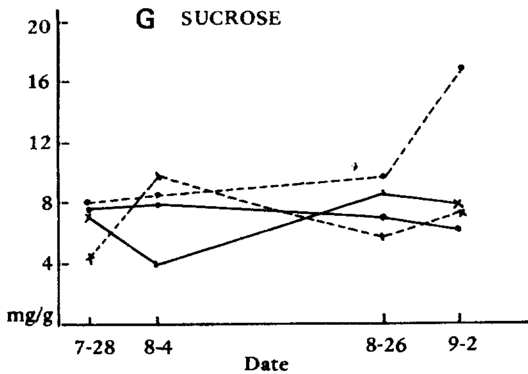


Fig. 3. Soluble (ethanol-soluble) sugar contents (mg/g dry weight) in 4 types of terminal buds collected in 1976 from abundant- and poor-flowering groups of slash pine. Sugars were determined by gas-liquid chromatography as described in the text. Total sugars mean a sum of individual soluble sugars.

Line identities:

●———●: abundant-flowering, large bud

✕———✕: abundant-flowering, small bud

●-----●: poor-flowering, large bud

✕-----✕: poor-flowering, small bud

Amino Acid Content in the Terminal Buds :

Fig 4 shows the results of amino acid analysis. In *Pinus elliottii* buds, 17 different amino acids commonly found in plant proteins were detected. An amino acid which eluted between serine and glutamic acid was found in small quantity in most of the sample, but it was not identified.

The amino acids listed below were detected less than 0.2 μmole/g and were not included in Figure 4: histidine, cystein, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine. However, the quantities of histidine, cystein, valine, isoleucine, and leucine were included in the calculation of the total amino acids (Fig. 4 A).

In all four types of buds, arginine was the most abundant amino acid. The relative concentration of arginine to total amino acids in overall average of four buds increased with time from 23.3% on July 28 to 29.0% on August 4, 47.9% on August 26, and 60.2% on September 2. It indicated the accumulation of arginine in all four types of buds. During the observation period, individual amino acids varied considerably. However, there were certain patterns of changes: basic amino acids (positively charged), lysine and arginine increased over the sampling period, while acidic amino acids (negatively charged), aspartic acid and glutamic acid suddenly decreased at the end of the sampling period.

In Figure 4 A, total amino acid content increased considerably during the sampling period, with an exception of the large buds of AFG (female-producing buds). In those particular buds the concentration of total amino acids remained virtually the same during the sampling period and were lower than the other three types of buds in the last three sampling dates. When large and small buds of AFG were compared, small buds contained about four times more total amino acids than large buds on September 2.

During the last three sampling dates, large buds of AFG contained lower concentration of lysine and arginine than other three types of buds. The low total amino acid concentration in large buds of AFG (female-producing buds) resulted mainly from the low concentration of arginine.

Sugar to Amino Acid Ratio :

Fig 5 shows the amount of free sugars relative to amino acid content (the ratio of sugars to amino acids). The ratio in the large buds of AFG was significantly higher than in the other three types of buds during the last three sampling dates. Due to increased concentration of amino acids during the last three sampling dates, the ratio in the all four types of buds decreased with time.

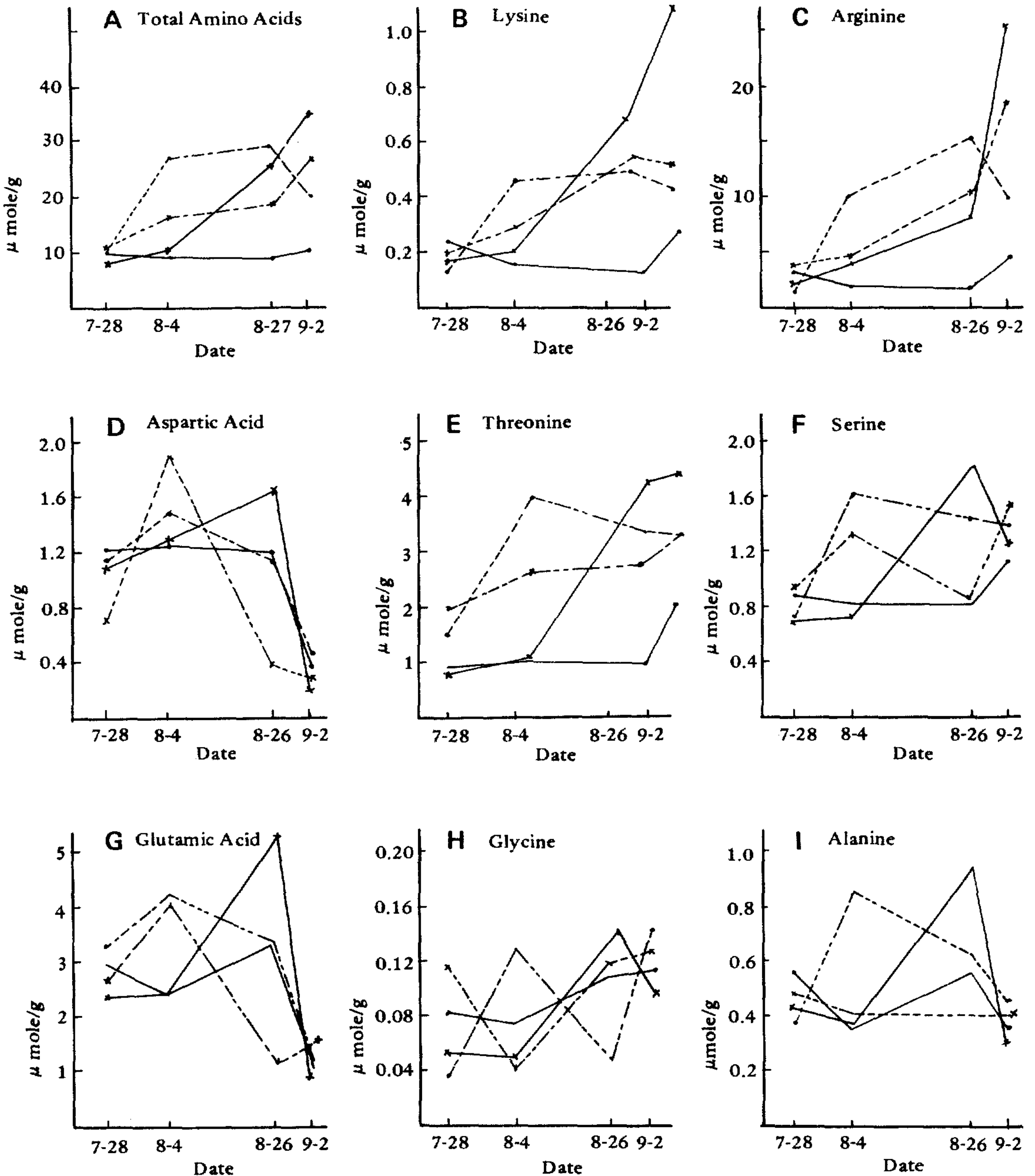


Fig. 4. Free amino acid (75% ethanol-soluble) contents in four types of terminal buds collected in 1976 from abundant-flowering and poor-flowering groups of slash pine. Amino acids were determined by an automatic amino acid analyzer as described in the text. Total amino acids mean a sum of individual amino acids in μ mole/g of dry weight.

Line identities:

●———● : abundant-flowering group, large bud

×———× : abundant-flowering group, small bud

●- - - -● : poor-flowering group, large bud

×- - - -× : poor flowering group, small bud

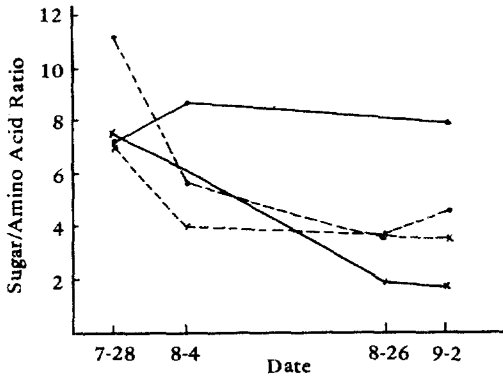


Fig. 5. Ratio of free sugars (ethanol-soluble) to free amino acids (ethanol-soluble) in the four types of the terminal buds from abundant-flowering and poor-flowering groups of *Pinus elliottii* during the period fo female flower initiation. Sugars were determined by gas-liquid chromatography and amino acids by an automatic amino acid analyzer.

Line identities:

•———• : abundant-flowering group, large bud

×———× : abundant-flowering group, small bud

•------• : poor-flowering group, large bud

×------× : poor-flowering group, small bud

DISCUSSION

The greater amounts of soluble sugars in the large buds of both flowering group indicated that large buds surrounded by larger number of needles than small buds received greater amounts of carbohydrates from the surrounding needles than small buds. However, due to no difference in the sugar contents between female-producing (large buds of AFG) and vegetative buds (large buds of PFG), sugar data alone failed to show the specific condition of female-producing buds during the period of floral bud initiation.

Results of amino acid analysis in which female-producing buds contained smaller amounts of amino acids, especially arginine, than three other types of buds suggested that arginine might play a minimal role in female flower initiation. Barnes and Bengtson (1968) observed increased free amino acids, especially arginine in needles after nitrogen fertilization. Ebell and McMullan (1970) proposed that arginine-type metabolism was quantitatively associated with female cone production. Sweet and Hong (1978) also observed the correlation between free arginine in buds and female cone production. The present study, however, showed low level of arginine in the female-producing buds during the period of floral bud initiation. Accumulation of arginine in their studies may simply reflect the abundance of nitrogenous compounds as a storage form of excess nitrogen after heavy fertilization. Arginine accumulation appears to be quite common in pine species (Ebell, 1969; Durzan and Steward, 1967), and may not necessarily be related to female flowering.

Kozlowski (1971) suggested that a higher nutritional status was required for development of female cones than for male cones. It appears that the relative availability of carbohydrates to nitrogen, rather than absolute amounts of carbohydrates alone, might be a better indication of the nutritional status of the tree. As shown in the present study, female-producing buds maintained a significantly higher C/N ratio than vegetative or male-producing buds. This high C/N ratio was primarily due to the low amino acid contents. This indicated that female flower initiation was associated with a relatirely low supply of nitrogen during the time when the supply of carbohydrates was not limiting. Such situation may lead the shoots to a "nutritional stress" which is responsible for the transition of the meristem-to a reproductive phase. Flowering of some conifers has been reported to be associated with high C/N ratio which was caused by nitrogen starvation (Kuo, 1973; Kamienska *et al.*, 1973; Kyr and Hoffmann, 1964), gibberellin

treatment (Hashizume, 1961), and girdling (Hashizume, 1970).

This nutritional stress mentioned above appears to be caused by many cultural treatments that have been known to stimulate female flowering in conifers. The nitrogen fertilization that caused "critically timed changes in type of nitrogen metabolism" (Ebell, 1972, P. 317), moisture stress in summer (Shoulders, 1973), girdling (Ebell, 1971), thinning, and root pruning seems to induce sudden changes in the nutritional status of the tress. And this disturbance in the nutritional balance may cause shift to a reproductive phase (Lee, 1979), and appears to the writer to be related to gibberellin metabolism. Kuo (1973) and Pharis (1976, 1977) reported promotion of flowering and at the same time increase in the endogenous levels of less-polar gibberellins by induced nitrogen deficiency or nitrate fertilization. Increased levels of less-polar gibberellins in water-stressed or girdled trees (Pharis, 1976, 1977) also support the idea that gibberellins may play a role in measuring the balance between carbohydrates and nitrogen by means of interconversion of gibberellins. More specifically, Pharis and Kuo (1977) postulated that one role of gibberellins in inducing flowering was by allowing for enhanced mobilization of photosynthate to the meristem where flower buds were initiated.

LITERATURE CITED

Barnes, R.L. and G.W. Bengtson 1968. Effects of fertilization, irrigation, and cover cropping on flowering and on nitrogen and soluble sugar composition of slash pine. For. Sci. 14:172-180.

Durzan, D.J. and F.C. Steward 1967. The nitrogen metabolism of *Picea glauca* (Moench) Voss and *Pinus banksiana* Lamb. as influenced by mineral nutrition. Can. J. Bot. 45:695-710.

Ebell, L.F. 1969. Biochemical aspects of cone production in Douglas fir. In Proc. 11th Internat. Bot. Congress, Seattle, 1969.

Ebell, L.F. 1971. Girdlings: its effect on carbohydrate status and on reproductive bud and cone development in Douglas fir. Can. J. Bot. 49:453-466.

Ebell, L.F. 1972. Cone-induction response of Douglas-fir to form of nitrogen fertilizer and time of treatment. Can. J. For. Res. 2:317-326.

Ebell, L.F. and E.E. McMullan 1970. Nitrogenous substances associated with differential cone production responses of Douglas fir to ammonium and nitrate fertilization. Can. J. Bot. 48:2169-2177.

Ford, C.W. 1974. Semimicro quantitative determination of carbohydrates in plant material by gas-liquid chromatography. Anal. Biochem. 57:413-420.

Fowells, H.A. and G.H. Schubert 1956. Seed crops of forest trees in the pine region of California. U.S.D.A. Tech. Bull. No. 1150, 48 p.

Fretz, T.A., C.W. Dunham and C.W. Woodmansee 1970. A gas chromatographic procedure for determining soluble carbohydrates extracted from leaf tissues of *Ilex opaca* Ait. Cv. 'Miss Helen'. J. Amer. Soc. Hort. Sci. 95:99-102.

Hashizume, H. 1961. The effect of gibberellin on flower bud formation in *Cryptomeria japonica*. III. Changes of endogenous growth substances, carbohydrates and nitrogen in new shoots in relation to flower induction by gibberellin. J. Jap. For. Soc. 43:120-126.

Hashizume, H. 1970. Effect of mechanical treatments on flower bud formation in conifers and change of nutrients in shoots in relation to flower induction. Bull. Faculty Agric., Tottori Univ. 22:25-33.

Kamienska, A., R.P. Pharis, R.L. Wample, C.G. Kuo and R.C. Durley 1973. Gibberellins in conifers. In Plant Growth Substances (S.

Tamura, ed.) 305-313. Hirokawa Publ. Co. Tokyo, Japan.

Kozlowski, T.T. 1971. Growth and Development of Trees. Vol. II. Academic Press, New York and London, 514p.

Kramer, P.J. and T.T. Kozlowski 1960. Physiology of Trees, McGraw-Hill Co., N.Y., 642p.

Kraus, E.J. 1925. Soil nutrients in relation to vegetation and reproduction. Amer. J. Bot. 12:510-516.

Kuo, C.G. 1973. Growth retardation and nutritional stress in relation to vegetative growth and reproductive differentiation of seedlings of *Cupressus arizonica* Greene. Ph.D. Dissertation, Univ. Calgary, Alberta, Canada, 157p.

Lee, K.J. 1979. Factors affecting cone initiation in pines: A review. Res. Rep. Inst. For. Genet., Korea 15:45-85.

Lee, K.J. 1980. Physiology of strobilus initiation in slasp pine I. Ovulate strobilus production in relation to shoot morphology. J. Korean For Soc. 46:21-28.

Lyr, V.H. and G. Hoffmann 1964. Über den Einsfluss der Mineralsalzernahrung auf die Frühfruktifikation von *Crytomeria japonica* (L.F.D.) Don. Flora 154:189-208.

Pharis, R.P. 1976. Manipulation of flowering in conifers through the use of hormones. In Modern Methods in Forest Genetics. (ed. J.P. Miksche) Springer-Verlag Berlin, 265-282.

Pharis, R.P. 1977. Interaction of native or exogenous plant hormones in the flowering of woody plants. In "Regulation of Developmental Processes in Plants" (eds. H.R. Schutte and D. Gross) Proc. Conf. held at Halle, July 4-9, 1977. 343-360.

Pharis, R.P. and C.G. Kuo 1977. Physiology of gibberellins in conifers. Can. J. For. Res. 7: 299-325.

Shoulders, E. 1973. Rainfall influences female flowering of slash pine. U.S.D.A. For. Serv. Res. Note SO-150.

Splittstoesser, W.E. 1969. Arginine metabolism by pumpkin seedlings: Separation of plant extracts by ion exchange resins. Plant Cell Physiol. 10:87-94.

Sweet, G.B. and S.O. Hong 1978. The role of nitrogen in relation to cone production in in *Pinus radiata*. N.Z.J. For. Sci. 8:225-238.