〔韓國林學會誌 No. 48, P. 51～58(1980) (論文)〕

슬래쉬소나무의 花芽原基 形成의 生理学的 研究
Ⅲ. 雌花原基形成期間동안 ^{14}C－光合成物質의 頂芽로의 移動*1

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Physiology of Strobilus Initiation in Slash Pine
Ⅲ. Translocation of ^{14}C－photosynthate to Terminal Buds during the Period of Female Strobilus Initiation *1

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雌花의 原基形成期間 동안에 光合成物質(Photosynthate)이 잎으로부터 頂芽로 移動하는 様相을 究明하기 위하여, 放射能 同位元素 $^{14}CO_2$를 잎에 處理하여 放射能의 移動을 追跡하였다. 7月末과 8月末 두 차례에 걸쳐서 当年枝의 잎에 $50_{\mu}Ci$의 $^{14}CO_2$를 90分間 處理한 후, 1日과 7日后에 그当年枝를 採取하여 放射能 을 測定했다. 處理 1日후에는 頂芽内 總放射能의 85% 以上이, 7日후에는 그의 55% 정도가 에칠알콜 可溶 Fraction에서 檢出되었다.

8月末에 $^{14}CO_2$를 處理했을때, 雌花를 生産하는 頂芽로 移動된 放射能物質의 量은 雄花를 生産하는頂芽와 營養상태에 머물러 있는 頂芽로 移動된 量보다 훨씬 적었다. 에칠알콜 可溶 Fraction을 hexane可溶, 아미노산, 糖類, 有機酸 Fraction으로 細分하였을 때, 雄花를 生産하는 頂芽는 雌花를 生産하는 頂芽보다 그 放射能 物質의 量이 각 Fraction에서 3倍가량 많았다. 이것은 雄花를 生産하는 頂芽는 7月에 이미 形成된 原基의 分化로 因하여, 8月末에 多量의 光合成物質을 要求하게 된다는 것을 意味한다. 反面에 雌花를 生産하는 頂芽는 原基形成 期間인 8月末경에 光合成 物質을 별로 要求하지 않으며, 代身 一時的인 新陣代謝 活動의 減少를 同伴하는 것 같이 생각된다.

Translocation of ^{14}C－labelled photosynthate from needles to terminal buds of *Pinus elliottii* was studied to understand a pattern of mobilization of photosynthate to buds during the period of female strobilus initiation.

Current-year shoots of 20-year-old trees were exposed in late July and late August to 50 μ Ci of $^{14}CO_2$ for 90 minutes, and harvested 1 day and 7 days after the exposure to analyze distribution of ^{14}C in ethanol-soluble and ethanol-insoluble fractions. Over 85% of total radioactivity of bud tissue one day after the exposure and 55% total activity seven days after the exposure remained in the ethanol-soluble fraction.

The amount of ^{14}C translocation into female-producing buds in late August was significantly less than into vegetative and male-producing buds both one day and seven days after the exposure. When the ethanol-soluble fraction was further fractionated into hexane-soluble, amino acid, sugar, and organic acid fractions, radioactivity in the female-producing buds in late August was about a third of that in male-producing buds in all the fractions. This indicated that the male-producing buds required in late August but not in late July mobilization of large amount of photosynthate for developing male strobilus primordia. Female-produc-

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ing buds, on the other hand, did not mobilize as much photosynthate as male-producing buds in late August when female strobilus primordia are believed to be initiated. It is suggested that initiation of the female strobilus primordia is associated with temporary reduction in the metabolic activity of the terminal buds in slash pine.

INTRODUCTION

Initiation and subsequent development of floral structures in trees require mobilization of carbohydrates from photosynthesizing leaves to the site of floral development (Zimmermann, 1967). In pines one-year-old needles were primary exporters of ^{14}C-labelled photosynthate to the currently expanding shoots and cones (Dickmann and Kozlowski, 1968;1970). The significance in cone production of the amount of light received by individual trees (as shown by stimulation of cone production by thinning) suggests that photosynthate from the needles contributes to the cone initiation process.

Translocation of photosynthate labelled with ^{14}C has been studied in young pine tress in relation to vegetative growth of shoots and roots (Ursino etal., 1968; Rangnekar and Forward, 1969; Ziemer, 1971). However little is known about translocation and subsequent metabolism of photosynthate from needles to terminal buds of adult tress during the period of floral bud initiation. As described in previous paper (Lee, 1980b), female-producing buds of *Pinus elliottii* showed higher concentration of sugars than male-producing buds, suggesting that initiation of female strobili took place under the influence of an abundance of carbohydrates.

I report here, however, a slow rate of translocation of ^{14}C-photosynthate to the the female producing buds during the period of female strobilus initiation. The reason appears to be temporary reduction in the metabolic activity of terminal buds and demand of rapidly expanding sencond-year cones for photosynthate during the floral initiation peroiod.

MATERIALS AND METHODS

The slash pine (*Pinus elliottii* var. *elliottii* Engelm.) seed orchard used in this study was established in 1956 with grafted seedling, and 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, 1980a). In the abundant-flowering group (AFG), two types of terminal buds were recognized : 1) large buds in the upper part of tree 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.

Four types of branches representing four types of terminal buds as described above were exposed to $^{14}CO_2$. Only current-year shoots were exposed and care was taken to choose branches receiving full sunlight at the time of exposure to $^{14}CO_2$.

Preparation of $Na_2{}^{14}CO_3$ Solution : Sodium carbonate solution, which had a specific activity of 59mCi / mmol and a radioactive concentration of 5.0mCi/ml of aqueous solution, was diluted 10 times phosphate buffer (Na_2HPO_4 and KH_2PO_4 at PH 8.0) to give a radioactive concentration of 0.5μCi/μl.

Exposure to $^{14}CO_2$: $^{14}CO_2$ was generated by a method described by Dickmann and Kozlowski (1968) A small glass vial containing 3ml of 2M lactic acid was tied to a shoot. The current-year shoot was then enclosed in a heavy polyethylene bag and the open end of the bag was firmly tied around the base of the shoot. Hundred μl of sodium carbonate solution (50μCi in total radioactivity) was injected into the vial with a microsyringe. The shoots were allowed to assimilate $^{14}CO_2$ for 90 minutes, after which the bags were removed. The above treatment was carried out during the morning hours to avoid heat injury on July 28 and August 26. Six branches each for four types of buds were exposed to $^{14}CO_2$. Three branches were harvested 24 hours after the exposure, and the remaining three branches were harvested seven days after the exposure.

Determination of Radioactivity: Current- year needles (which expanded fully at the time of exposure) and a terminal bud from each harvested shoot were saved for the analysis. Needles and buds were separately freeze-dried and ground. About 100mg of duplicate samples of needles and buds was combusted by a sample oxidizer (Intertechnique IN 4101 L. S.) and the radioactivity in it was determined by liquid scintillation spectrometry.

The ground buds were extracted with 75% ethanol for free sugars (step"V"), amino acids) step"S"), and organic acids (step"W" in Figure 1.(The method of extraction was described in the second paper of this series (Lee, 1980b) and is summarized in Figure 1. Radioactivity in the various fractions was measured by withdrawing 1 ml aliquot, mixing with 10 ml Aquasol -2, and subsequent counting by liquid scinitillation spectrometry. Radioactivity left in the bud tissue after alcohol extraction (ethanol-insoluble fraction) was determined following combustion of the tissue.

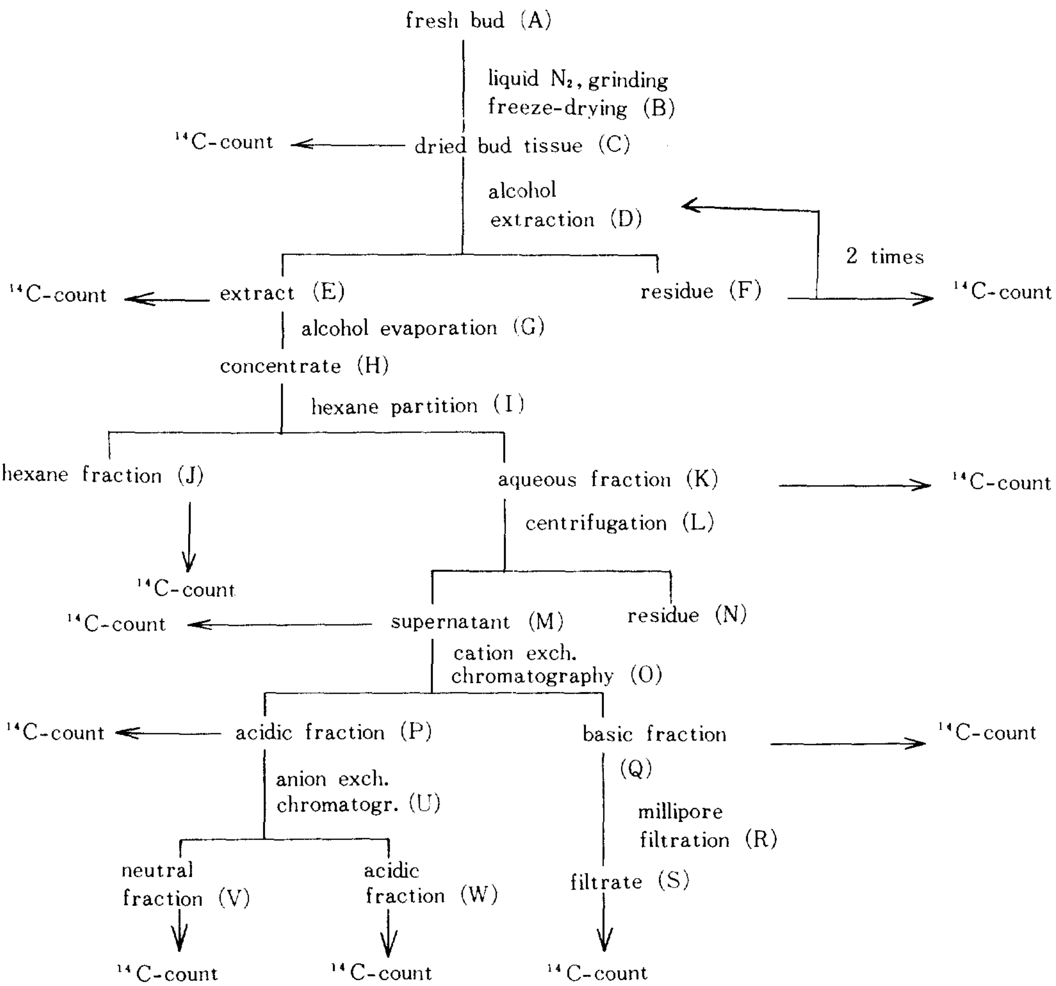
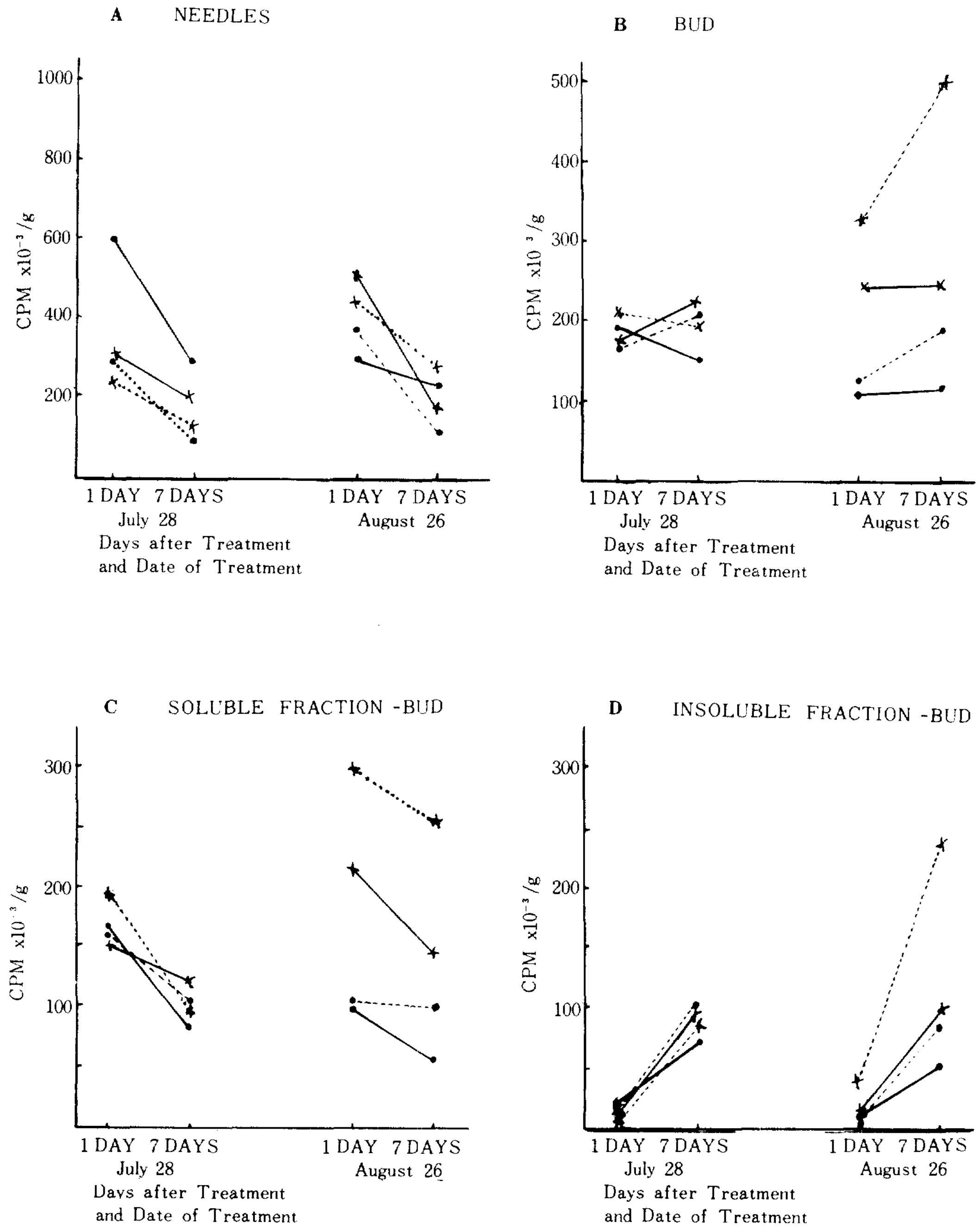


Fig. 1. Extraction procedures for free sugars, organic acids, and free amino acids and determination of radioactivity in their fractions of ***Pinus elliottii*** bud tissue.

RESULTS

Figure 2 shows the distribution of radioactivity in needles, buds and various fractions of bud extract. Radioactivity in the needles (Fig. 2A) of the large buds of AFG during the first exposure on July 28 was greater than in the needles of the three other types of buds, but it was reversed in the second exposure on August 26. Radioactivity in the needles decreased considerably seven days after the exposure, indicating the export of ^{14}C-photosynthate from needles to other parts of the shoots.



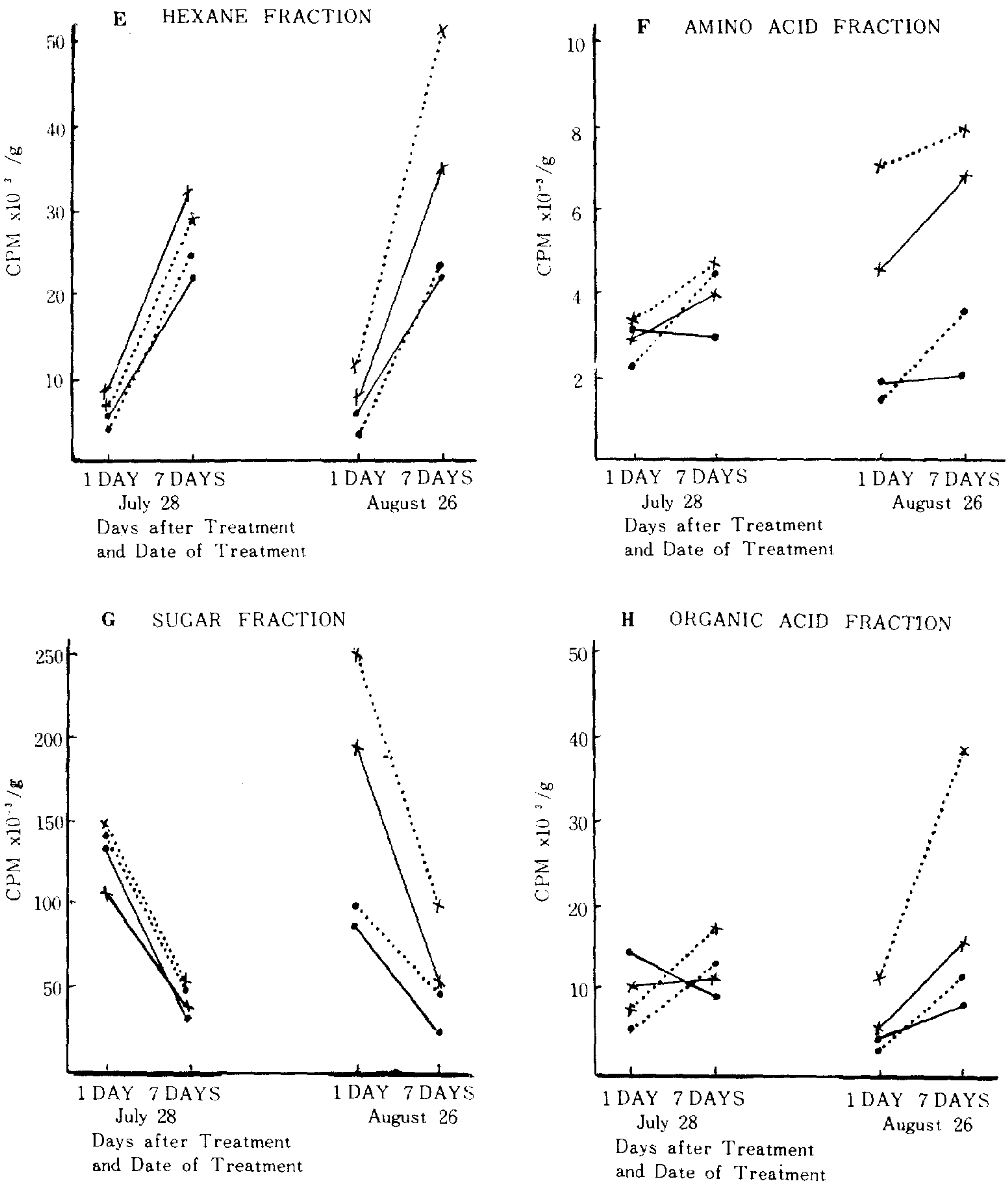


Fig. 2. Distribution of radioactivity in needles, *bud*, and various fractions of bud extract from abundant-flowering and poor-flowering groups of *Pinus elliottii* 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. Each point is an average of two observations from two different trees.

Line identities:

●————● : abundant-flowering group, large bud
×————× : abundant-flowering group, small bud
●··········● : poor-flowering group, large bud
×- - - - - - - -× : poor-flowering group, small bud

The bud tissue (Fig. 2B) during the first exposure showed no difference in radioactivity among the four types of buds, whereas radioactivity in the small buds of both flowering groups during the second exposure (on August 26) was much higher than in the large buds of either group. The small buds of PFG showed about 3 times more radioactivity than large buds of AFG which contained lowest radioactivity among four types of buds.

When radioactivity in the bud tissue was divided into ethanol-soluble and ethanol-insoluble fractions (Fig. 2C and 2D), over 85 % (an overall average of four types of buds) of total activity one day after exposure and 55% of activity seven days after exposure remained in the ethanol-soluble fraction (also shown in Table 1). Radioactivity of the ethanol-soluble fraction in the small buds during the second exposure on Aug. 26 was greater than in the large buds of either flowering group (Fig. 2C).

The ethanol-soluble fraction was further fractionated into hexane-soluble, amino acid, sugar, and organic acid fractions (Fig. 2E through 2H). Radioactivity in sugars, the primary transporting agent, decreased over the seven-day period, while activity in the hexane fraction, amino acids, and organic acids increased during the same period with the exception of amino acids and organic acids of large

Tab. 1. Distribution of radioactivity (percent of total radioactivity of bud) in ethanol-soluble, hexane-soluble, amino acid, sugar, and organic acid fractions of bud extract from abundant-flowering (AFG) and poor-flowering groups (PFG) of *Pinus elliottii* 1 day and 7 days after current-year needles were exposed to 50 μCi of $^{14}CO_2$ for 90 minutes. The ethanol-soluble fraction was fractionated into hexane-soluble, amino acid, sugar, and organic acid fractions.

fraction	flowering group	bud size	date of treatment			
			July 28		August 26	
			1 day	7 days	1 day	7 days
ethanol-soluble	AFG	large	89.5	53.3	90.9	50.0
	PFG	large	84.2	70.9	84.6	54.1
	AFG	small	88.2	53.3	91.7	59.2
	PFG	small	92.8	51.3	90.9	54.2
hexane-soluble	AFG	large	2.6	15.0	5.5	19.2
	PFG	large	2.4	12.2	2.4	12.9
	AFG	small	4.6	15.0	3.1	14.0
	PFG	small	3.3	14.9	3.6	10.8
amino acid	AFG	large	1.7	2.0	1.6	1.7
	PFG	large	1.4	2.2	1.1	1.9
	AFG	small	1.6	1.8	1.8	2.7
	PFG	small	1.6	2.5	2.1	1.6
sugar	AFG	large	68.4	20.0	75.4	18.3
	PFG	large	84.8	21.9	80.0	23.2
	AFG	small	62.9	15.9	77.6	22.0
	PFG	small	69.0	25.6	75.8	20.8
organic acid	AFG	large	7.9	6.0	3.6	6.7
	PFG	large	3.0	6.7	2.4	6.5
	AFG	small	5.7	5.0	2.0	6.4
	PFG	small	3.3	8.7	3.5	7.9

buds of AFG in July (Fig. 2F and 2H). Radioactivity in various fractions of the small buds were at all times higher than in those of the large buds of either flowering group.

The radioactivity in the various fractions was expressed as a percent of total radioactivity of bud (Table 1). Little difference was noticed among the four different types of terminal buds at either treatment period. However, hexane-soluble fraction of large buds of AFG on Aug. 26 treatment showed higher radioactivity percent (5.5 and 19.2%) than that of three other types of buds.

DISCUSSION

The greater radioactivity (per g dry weight) in the small buds of both flowering groups than in the large buds (Fig. 2B) suggests that the small buds act as active sinks for photosynthate in late August and early September when male strobilus primordia are under active differentiation. The small buds of PFG which produce greater number of male strobili than the small buds of AFG (as described in Lee, 1980a), were also stronger sinks than the small buds of AFG. This suggested that there was a quantitative relationship between the number of developing male strobilus primordia and the amount of photosynthate translocated into the bud.

The lowest radioactivity (per g dry weight) in the female-producing large buds of AFG indicates that the capacity of these buds to act as metabolic sinks might be reduced during the period of floral initiation. These buds do not initiate male strobilus primordia and, thus, do not require in late August as much photosynthate as the small buds.

According to Lanner (1976), slash pine features in shoot development "free growth" (elongation of a shoot due to the simultaneous initiation and elongation of new stem units) as well as "fixed growth" (elongation of predetermined stem units). The last free growth, if more than one, in a growing season is followed by termination of elongation growth and reduction in metabolic activivy of the terminal buds prior to the initiation of female strobili. The low radioactivity in the female-producing large buds of AFG as shown in the present study directly reflects the low metabolic activity of these buds. This point is further supported by the low amino acid contents in the large buds of AFG during the floral initiation period (Lee, 1980b). Temporary cessation of any activity in the apical meristem appears to be the first effect of photo-induction in many herbaceous plants and was followed by transition from vegetative growth to flower initiation (Lang, 1965).

Another explanation for the low radioactivity in the female-producing buds is primary transfer of photosynthate to developing second-year cones on the same shoots. Actively elongating cones (second-year cones in the large buds of AFG in the present study) were shown to be the strongest sink for ^{14}C-photosynthate (Dickmann and Kozlowski, 1968, 1970). Thus, large buds of AFG seemed to receive less amount of ^{14}C from needles than the small buds.

The present study of ^{14}C translocation did not directly answer the question of how ^{14}C-photosynthate and its metabolites are involved in the initiation of female strobilus primordia. However, based on the previous (Lee, 1980b) and present studies, it is concluded that the initiation of female strobilus primordia is preceded by strong vegetative growth in the early growing season and by following temporary reduction in the metabolic activity of the terminal buds in the mid summer. The requirement of temporary cessation of metabolic activity for initiation of female stobili coincides with stimulation of female cone production by various silvicultural treatments and certain weather conditions which are believed to induce severe shock or temprorary cutback in vegetative growth (Schmidtling, 1974; Lee, 1979).

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