Seasonal Variation of Arginine in Buds of *Pinus radiata* in Relation to Flower Initiation*[1]

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Pinus radiata 소나무의 頂芽內 Arginine含量의 時期的變化*[1]

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SUMMARY

Seasonal changes in free arginine concentration in *Pinus radiata* buds were studied in four clones from May, 1974 to June, 1975. The buds were from the tips of first order branches which had flowered the previous year, and the significant findings were as follows:

(1) In general, arginine concentration in the buds of the four clones showed the highest peaks from December to March, a period spanning the time of flower initiation, and the lowest levels in August and September.

(2) The timing of individual arginine peaks during the period of flower initiation matched roughly the timing of initiation of several female cone clusters, as determined from Clone 7 which characteristically initiates three cycles of female cones on its leading shoot.

(3) The heavy-flowering clones showed higher arginine concentration than ther poorer flowering ones, especially at the time of flower initiation.

本 實驗에서는 *Pinus radiata*의 頂芽內 遊離 arginine含量의 時期的 變化를 花芽分化와 關聯하여 1974年 5月부터 1975年 6月까지의 期間에 걸쳐 硏究하였다. 使用된 頂芽는 뉴질랜드 로토루아市 所在 林業硏究所 圃場에 生長하고 있는 4個의 Clone에서 採取하였으며, 前年度에 開花하였던 가지의 頂芽를 arginine 分析에 利用하였다. 本 硏究의 主要한 結果는 다음과 같다.

1. 一般的으로 頂芽內 arginine의 含量은 4個 Clone에서 共히 花芽分化期인 12月~3月(北半球의 6月~9月에 該當)에 絕頂에 達하였고 開花期인 8月~9月에 가장 低調하였다.

2. 花芽分化期中 arginine이 絕頂에 達하는 回數는 各 Clone의 雌花分化의 週期數(*P. radiata*에서는 1~3週期인)와 大體로 一致하였다.

3. 開花量이 많은 Clone은 적은 Clone에 比하여 높은 arginine含量을 나타내었고 이러한 現象은 特히 花芽分化期에 더욱 顯著하였다.

4. 그러므로 頂芽內 arginine의 含量은 花芽分化와 密接한 關係가 있다고 思料된다.

INTRODUCTION

The free amino acid arginine has been studied as a major component of stored nitrogen in horticultural plants(Taylor, 1967; Tromp, 1969). Recent investigations have shown that arginine is one of the metabolites which was significantly increased, along with female cone initiation, by appropriate nitrogen fertilization; both in Douglas fir (Ebell & McMullan, 1970;

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Ebell, 1972; Ching *et al.*, 1973), and in American southern pines (Barnes & Bengtson, 1968a, b; Stanley & Smith, 1970), indicating that arginine may play a role in flower initiation(Jackson & Sweet, 1972). Arginine was found to be the most abundant amino acid in buds and conelets of pine (Barnes & Bengtson, 1968b) and in buds of spruce (Durzan, 1968).

Therefore, it was of interest to study in *P.radiata* whether arginine levels increase (in buds where reproductive primordia are induced) during the period of flower initiation as compared with the rest of the year. Few studies have examined arinine levels in relation to flowering in pine.

This paper reports how arginine concentration in buds of *P. radiata* fluctuated during the year, with special emphasis on the time of flower initiation. The study included a comparison of good and poor-flowering clones.

This study was carried out at Forest Research Institute, Rotorua, New Zealand.

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METHODS

Some considerations in sampling

In a previous experiment some questions of sampling arose, e.g. to what length should a terminal bud sample be preparded? And should lateral buds adjacent to the terminal be included (or not) in the sample for extraction of amino acids?

To answer these questions arginine concentrations were compared between different parts of a terminal bud and between a terminal bud and its laterals. For this purpose three buds having several laterals were collected on March 22, 1974 from Clone 121 planted in 1966 at Long Mile. A terminal bud was cut 3 cm long and divided into three parts of equal length (1 cm each), apex, middle and base. The latral buds attached to the terminal bud were bulked for extraction.

Arginine contents were determined by the routine methods described in the previous report(Hong & Sweet, 1974). The quantitative method of arginine analysis gave a coefficient of variation between 4% and 5% for ten determinations.

As shown in Table 1, arginine was concentrated more in the middle part of a terminal bud than in the apex or basal part of it; and arginine contents in terminal buds were about double those in laterals. In other words, three centimetres, the length of a bud sample, was enough to incorporate the main pool of amino acids in the bud; and it is better not to include the laterals in the bud sample because the number and size of these influences the arginine concentration of the terminal bud.

Table 1. Comparison of arginine concentrations in different parts of a terminal bud, and between the terminal bud and its lateral buds.

(arginine : μg/10mg fresh weight)

	Bud 1	Bud 2	Bud 3
Terminal			
apex	2.30	7.80	4.20
middle	9.50	14.00	11.00
base	8.50	10.00	8.70
Terminal*	7.63	11.05	8.72
Laterals	3.60	6.70	4.00
Mean**	5.60	9.20	6.31

* arginine content in the bud when the laterals were not included.

** mean arginine content in the bud if the laterals were bulked with the terminal bud.

Therefore, it was decided that, in the main experiment, a bud sample should be prepared to 3 cm in length and all laterals should be removed.

Main experiment

Four clones of *P. radiata* grafts planted at Long Mile in 1965 were used for this experiment. The number of cycles of conelets on the main leader and the flowering rank of the clones were as follows:

Clone	*No. of cycles of conelets on leader*	*Flowering rank.*
7	3	1
119	3	2
90	2	3
87	2	4

Terminal buds were harvested from conelet-bearing

first order branches of the four clones every three weeks from May 22 to July 24, 1974 and afterwards fortnightly until June 11, 1975; and stored in liquid nitrogen until used for amino acid extraction. The procedures of amino acid extraction and quantitative analysis of arginine were the same as mentioned above.

RESULTS

Seasonal variation in free arginine concentration, total amino acid concentration and arginine as a percentage of total amino acids in buds of four clones of *P. radiata* are shown in Table 2, 3 and 4, respe-

Table 2. Seasonal variations in free arginine concentrations in buds of *Pinus radiata* grafts.
(arginine : μg/10mg fresh weight)

Harvests	Clone 7	Clone 119	Clone 87	Clone 90	Mean	Remarks
May 22, 1974	8.14	3.73	0.92	3.97	4.19	
June 12	7.11	4.71	1.78	2.41	4.00	
July 3	6.05	2.38	2.55	1.18	3.04	
July 24	7.67	2.75	4.24	1.01	3.92	
Aug 8	4.53	1.67	1.53	1.30	2.26	
Aug 20	2.19	2.80	3.25	1.44	2.42	
Sept 4	0.51	0.41	1.70	1.00	0.91	
Sept 18	2.17	0.67	1.61	0.86	1.33	
Oct 2	3.82	1.39	2.68	2.25	2.54	
Oct 16	6.82	0.39	1.25	0.76	2.31	
Oct 30	11.52	3.29	3.25	3.08	5.29	
Nov 13	18.36	4.30	2.60	5.19	7.61	
Nov 27	10.79	6.05	7.36	6.13	7.58	
Dec 10	14.82	10.48	11.24	13.24	12.45*	peak
Dec 24	13.96	4.91	9.08	7.07	8.76	
Jan 10, 1975	10.73	3.00	9.27	4.85	6.96	
Jan 23	23.02	25.93	6.29	7.91	15.79*	peak
Feb 5	20.12	13.11	8.84	5.15	11.81	
Feb 19	12.60	7.48	8.31	4.20	8.15	
March 5	12.67	6.07	7.79	7.60	8.53	
March 19	16.15	12.20	11.07	8.81	12.06*	peak
April 2	8.04	9.18	2.92	8.22	7.09	
April 17	7.43	11.28	1.88	3.54	6.03	
May 1	11.72	2.70	7.27	2.78	6.12	
May 14	9.82	4.61	1.81	2.15	4.60	
May 29	8.36	3.84	1.55	6.21	4.99	
June 11	9.82	6.67	1.84	2.52	5.21	
Mean	9.96**	5.78	4.59	4.25		

* Significantly different from adjacent values at 5% level
** Significantly different from adjacent values at 1% level
LSD 0.05 between two harvests=4.05
LSD 0.05 between two clones=1.55
LSD 0.01 between two clones=2.07

Table 3. Seasonal variations in total amino acid concentrations in buds of *Pinus radiata* grafts. (μg/10mg fresh weight)

Harvests	Clone 7	Clone 119	Clone 87	Clone 90	Means	Remarks
May 22, 1974	29.26	18.32	9.47	18.53	18.89	
June 12	22.09	18.98	13.20	12.45	16.68	
July 3	29.93	19.42	14.14	11.69	18.79	
July 24	40.46	24.25	21.92	20.97	26.90	
Aug 8	30.21	16.20	20.17	13.91	20.12	
Aug 20	29.79	25.35	25.05	18.49	24.67	
Sept 4	21.75	18.46	26.60	21.43	22.06	
Sept 18	22.26	21.85	25.22	13.48	20.70	
Oct 2	27.07	19.72	22.09	22.84	22.93	
Oct 16	36.26	15.26	15.19	18.52	21.31	
Oct 30	39.06	17.60	26.65	24.82	27.03	
Nov 13	42.92	30.51	13.56	21.33	27.08	
Nov 27	29.24	23.20	26.86	28.93	27.06	
Dec 10	38.11	37.05	39.24	41.27	38.92	peak
Dec 24	29.04	19.89	28.44	25.10	25.62	
Jan 10, 1975	29.20	19.11	32.30	27.19	26.95	
Jan 23	47.31	68.22	32.07	33.51	45.28	peak
Feb 5	44.87	35.16	34.61	26.99	35.41	
Feb 19	32.84	31.22	32.98	27.13	31.04	
March 5	32.49	25.33	23.68	27.50	27.25	
March 19	39.79	32.64	29.79	30.39	33.15	peak
April 2	27.96	28.25	17.43	28.00	25.41	
April 17	20.79	26.87	10.92	16.38	18.74	
May 1	23.91	17.32	20.18	13.06	18.62	
May 14	23.20	15.36	14.37	14.66	16.90	
May 29	25.04	17.24	11.84	17.69	17.95	
June 11	36.61	33.47	13.87	10.85	23.70	
Mean	31.54	25.05	22.29	21.74		

ctively. The seasonal fluctuation of arginine in each clone, and that of the mean of the four clones are graphed in Figures 1 and 2, respectively. The arrows 1, 2, 3, 4 and 5 in two figures indicate the dates when five cycles of branch nodes were considered to be initiated on the main leader of Clone 7 at the Long Mile in 1973(Bollmann, unpublished). Female cone initiations occurred at the cycles marked by arrows 1, 2 and 3. As a reference monthly rainfall, mean temperature and number of dry days during the experimental period are shown in Figure 3.

In general, as Figure 2 shows, average arginine concentrations of all clones remained low(less than 10 μg/10 mg fresh weight) from May to July in winter and then gradually decreased to the lowest level (about 1 μg/10 mg fresh weight) from August to September at the start of spring. From October arginine concentrations in the buds increased rapidly and reached to three major peaks between December and March when female cone primordia were presumably initiated. The arginine concentrations at these major peaks measured on December 10, January 23, and March 19, respectively, were more than 10 μg/10 mg fresh weight and significantly higher than the adjacent ar-

Table 4. Seasonal variations in arginine percentages of total amino acids.

(%)

Harvests	Clone 7	Clone 119	Clone 87	Clone 90	Mean
May 22, 1974	27.82	20.36	9.71	21.42	19.83
June 12	32.19	24.82	13.48	19.36	22.46
July 3	20.21	12.26	18.03	10.09	15.15
July 24	18.96	11.34	19.34	4.82	13.62
Aug 8	15.00	10.31	7.59	9.35	10.56
Aug 20	7.35	11.05	12.97	7.79	9.79
Sept 4	2.34	2.22	6.39	4.67	3.91
Sept 18	9.75	3.07	6.38	6.38	6.39
Oct 2	14.11	7.05	12.13	9.85	10.79
Oct 16	18.81	2.56	8.23	4.10	8.43
Oct 30	29.49	18.69	12.20	12.41	18.20
Nov 13	42.78	14.09	19.17	24.33	25.09
Nov 27	36.90	26.08	27.40	21.19	27.89
Dec 10	38.89	28.29	28.64	32.08	31.98
Dec 24	48.07	24.69	31.93	28.17	33.22
Jan 10, 1975	36.75	15.70	28.70	17.84	24.75
Jan 23	48.66	38.01	19.61	23.60	32.47
Feb 5	44.84	37.29	25.54	19.08	31.69
Feb 19	38.37	23.96	25.20	15.48	25.75
March 5	39.00	23.96	32.90	27.64	30.88
March 19	40.59	37.38	37.16	29.02	36.04
April 2	28.76	32.50	16.75	29.36	26.84
April 17	35.74	41.98	17.22	21.61	29.14
May 1	49.02	15.59	36.03	21.29	30.48
May 14	42.33	30.01	12.60	14.67	24.90
May 29	33.39	22.27	13.09	35.10	25.96
June 11	26.82	19.93	13.27	23.23	20.81
Mean	30.63	20.57	18.95	18.29	

ginine concentrations before or after the peaks(Table 2). From April the arginine concentration decreased to a winter level which, from May to July, was almost the same as that of the previous winter.

Considerable clonal differences were found in the seasonal pattern of arginine changes of each clone, as seen in detail in Figure 1. Clone 7, which is the best flowerer among the four clones and has three clusters of female cones initiated on the leading shoot at the Long Mile, showed the highest concentrations of arginine through the year and four arginine peaks between mid November and mid March. Three of the four arginine peaks matched closely the likely initiation times* of the three cycles of female cone primordia on the leading shoot as the three arrows 1, 2 and 3 indicate in Figure 1. Arginine concentrations in buds were not so high when only vegetative long shoot primordia were initiated in early June and early August(refer to arrows 4 and 5).

Clone 119, a good flowerer having also three cycles of female cones initiated on the leading shoot, showed a similar trend in arginine changes to Clone 7 but

* Based on data collected at that site the previous year.

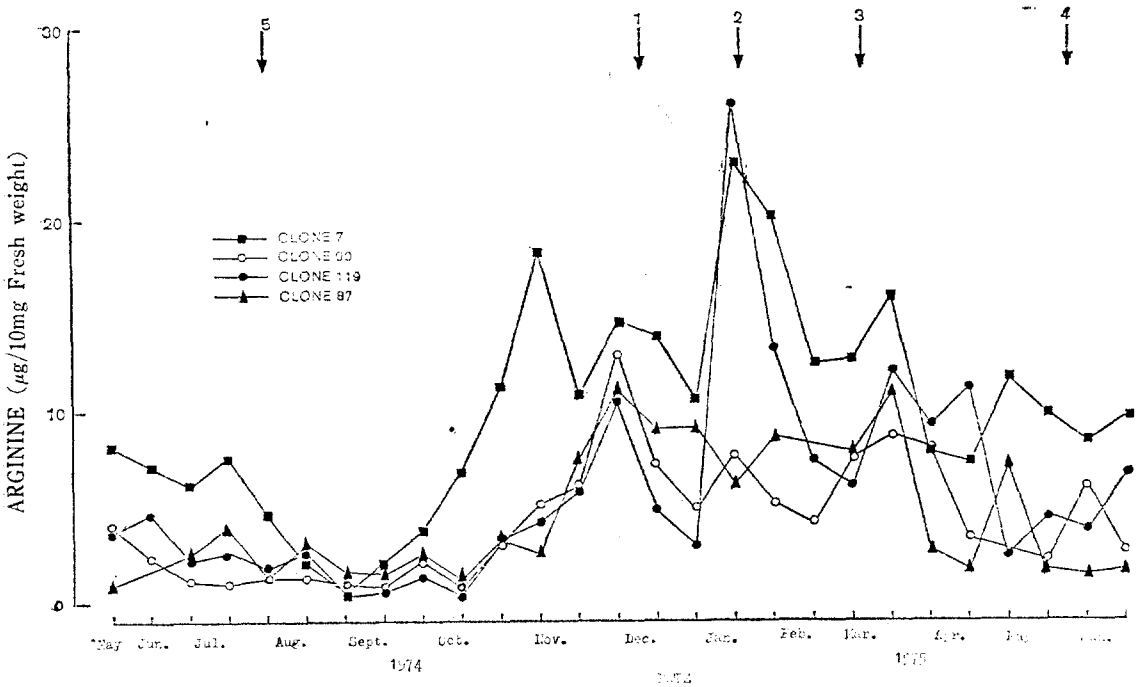

Fig. 1. Seasonal changes in free arginine concentration in buds of four clones of *Pinus radiata*. (Arrows indicate time of initiation of five branch clusters on leading shoot in Clone 7. Data are for the same site but for one year previously.
Arrows 1, 2 and 3 show the dates of first, second and third cycle of initiation of female cones and long shoots, respectively, on December 20, January 26 and February 27. Arrows 4 and 5 show the dates of fourth and fifth cycle of initiation of long shoots only.)

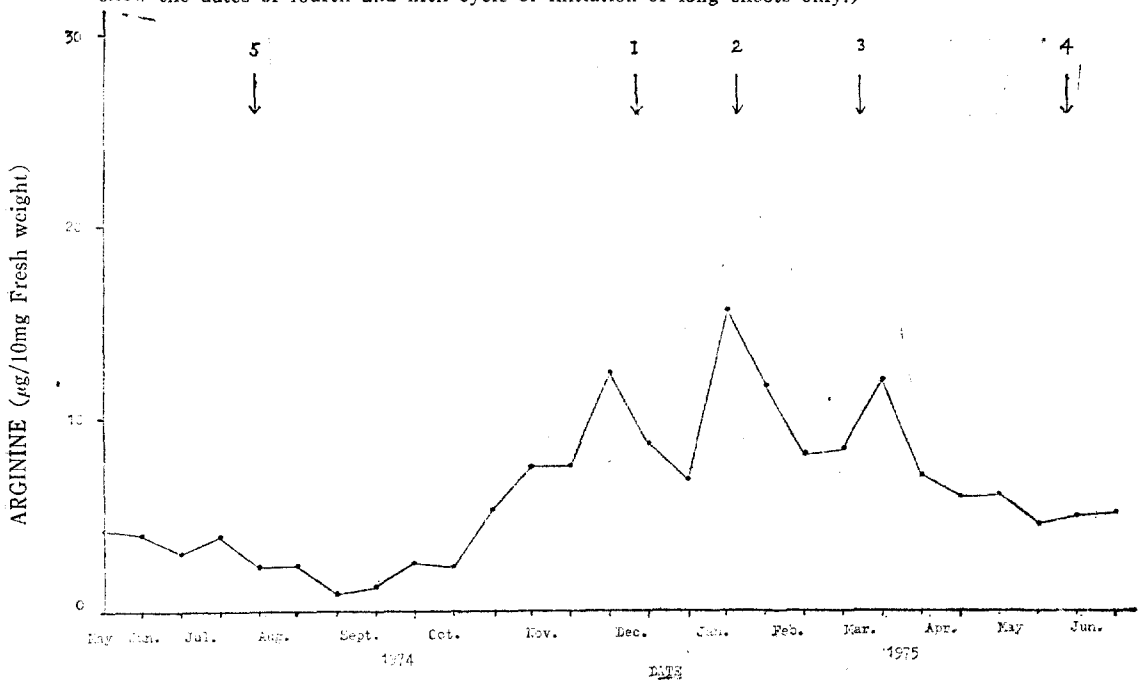

Fig. 2. Seasonal changes in mean arginine concentrations in the buds of four clones. The interpretation of the arrows 1—5 is as Figure 1.

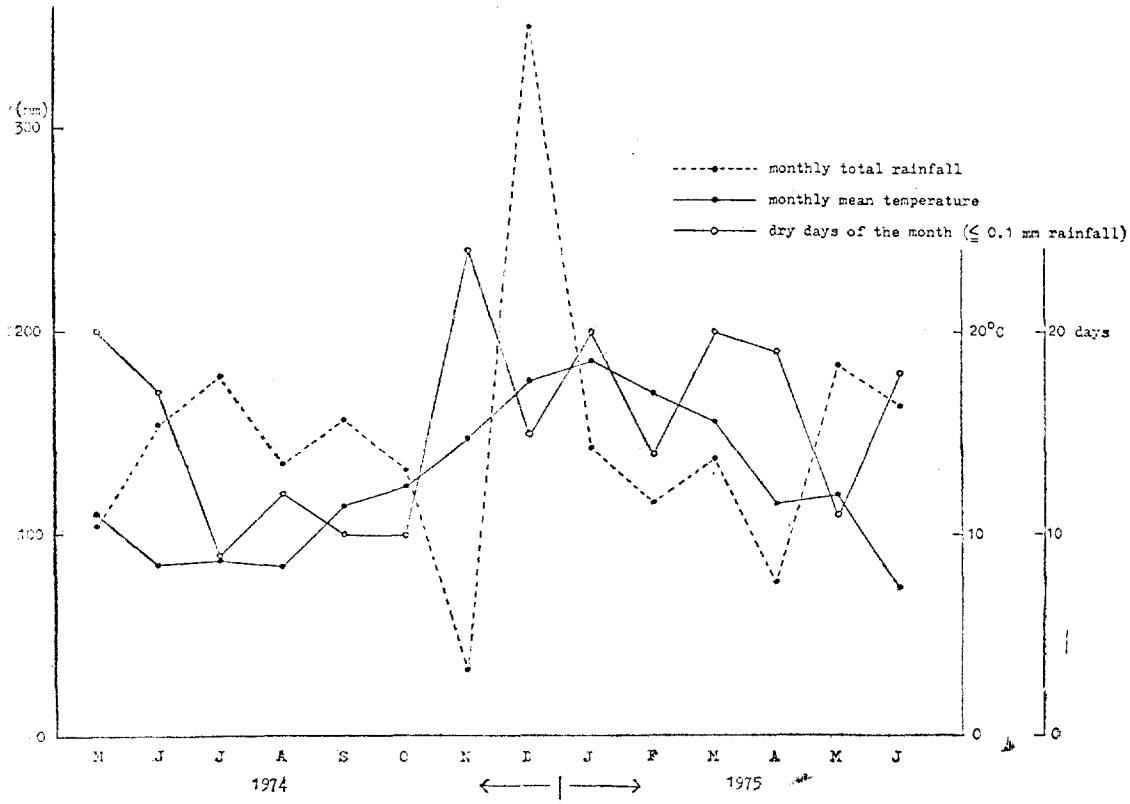

Fig. 3. Monthly rainfall, mean temperature and number of dry days from May, 1974 to June, 1975. Data are for FRI Met. Station, 1 kilometer from the Longmile area.

had only three arginine peaks during the period of flower initiation. It generally had lower arginine concentrations in the bud than Clone 7. The other two poorer-flowering clones 87 and 90, both having two cycles of female cone initiation, showed only two arginine peaks during the flower initiating period and also generally lower arginine concentrations than the better flowering clones.

Total amino acid concentrations and arginine levels expressed as a percentage of them mostly paralleled arginine concentrations. The arginine concentrations were high enough to influence appreciably the values of total amino acid and arginine percentage.

DISCUSSION

From the present study free arginine concentrations in buds of *P. radiata* gradually decreased from August as the spring started and become minimal in September during the period of pollination. The arginine increased from October and rapidly reached several peaks between December and March when female cone primordia were initiated. After the completion of flower initiation arginine concentrations decreased and remained at a relatively low level during the winter.

Such high increases in arginine were also reported in buds of *Picea glauca* in late summer when flower initiation of this species was believed to occur (Durzan, 1968). Stanley and Smith (1970) reported that extractable amino acids increased from spring to the induction period of reproductive buds in August (late summer) in *Pinus elliottii*.

Therefore, it seems to be a definite fact that high arginine contents in buds of many conifers are associated with flower initiation and/or flower determination. Though through which metablic pathway the

free amino acid arginine is synthesized so rapidly in bud at the time of flower initiation, and if utilised for the induction of reproductive buds how this happens, are not yet known.

A logical explanation is that the proteins associated with the reproductive, rather than general vegetative, development are arginine rich; and there is ample evidence that free arginine in the bud can be rapidly incorporated into protein(e.g. Durzan, 1968). But there is insufficient knowledge to make this general explanation other than speculative. Two of the six classes of histones are known to be arginine rich (Lewin, 1975) but so far, surprisingly, differences in histones have not been shown between vegetative and flowering shoots (Konstantinova *et al.*, 1974). It is known from organ and tissue culture studies that high arginine levels are associated with a reduced rate of mitotic activity (e.g. Chalupa & Durzan, 1973). There is wide evidence that woody plant flower initiation is associated with a check in general growth of the meristem, and thus any role of a high arginine level in the bud may be simply to reduce the general rate of bud activity, which in turn leads to flower initiation.

If it is true that a high arginine level is required to induce reproductive tissue primordia, it is important to know the least effective level of arginine contents in the buds to initiate flower primordia. From this experiment, the least effective level of arginine in *P. radiata* is thought to be about two milligrams in a bud of two grams fresh weight as the arginine concentration at the time of flower initiation was maintained mostly above the level. When vegetative buds only were initiated (arrow 4 and 5), arginine contents were relatively low. This fact suggests that arginine levels may not be important for vegetative bud initiation: however, this is by no means conclusive.

The ralationship between the number of peaks in arginine level in each clone, and the number of cycles of flowers initiated on the leading shoot is an interesting one. Its likely significance is somewhat diminished by the fact that the buds sampled for arginine were not from the leading shoots but from first-order laterals. It is also true that with only 2-weekly sampling, peaks may have been missed. Nonetheless the number of peaks in arginine concentration did reflect quite well the flowering capability of the clones in terms of number of cycles. The average arginine concentrations of each clone, both during flower initiation and throughout the year (Tables 5 and 2) also seemed to reflect flowering capacity of the clone. The better flowering clones, particularly Clone 7, showed significantly higher arginine concentrations than the other poorer flowering clones.

In conclusion, this experiment supports the belief that high arginine concentrations are required in a bud for flower initiation. Additionally, arginine analyses at the time of flower initiation may provide a

Table 5. Comparison of arginine concentrations in four clones during the period of flower initiation. (arginine : μg/10mg fresh weight)

Harvests	Clone 7	Clone 119	Clone 87	Clone 90	Mean
Dec 10, 1974	14.82	10.48	11.24	13.24	12.45
Dec 24	13.96	4.91	9.08	7.07	8.76
Jan 10, 1975	10.73	3.00	9.27	4.85	6.96
Jan 23	23.02	25.93	6.29	7.91	15.79
Feb 5	20.12	13.11	8.84	5.15	11.81
Feb 19	12.60	7.48	8.31	4.20	8.15
March 5	12.67	6.07	7.79	7.60	8.53
March 19	16.15	12.20	11.07	8.81	12.06
Mean	15.51*	10.40	8.89	7.35	

LSD 0.05 between clones=4.05

* Significantly greater than others at 5% level

criterion to characterise flowering behavior and capacity of clones of *P. radiata*.

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