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Rooting of Needle Fascicles of *Pinus radiata* in Test Tubes*1

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Pinus radiata 葉束挿木의 試驗管內 發根*1

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摘 要

라디아타 소나무의 葉束挿木을 試驗管內에서 實施하였던바 다음과 같은 結果를 얻었다.

1. 3年生, 7年生, 11年生, 18年生 및 40餘年生의 母樹別 平均 發根率은 各各 57%, 47%, 18%, 4% 및 2%이었으며 母樹令이 增加할수록 葉束挿穗의 發根率은 減少되었다.
2. IBA(indolebutyric acid), ABA(abscisic acid) 및 넷가지 殺菌劑의 處理는 發根에 別影響을 미치지 못하였다.
3. 溫度處理 20°/10°C(晝間溫度/夜間溫度)와 日照 18時間處理區의 葉束挿穗는 同溫度處理와 日照 10時間 處理區에서 보다 發根이 훨씬 좋았고 15°/10°C의 溫度處理와 10時間의 日照處理區에서 發根이 가장 不良하였다.
4. 試驗管內 葉束挿木의 發根率은 溫室內 發根率에 比하여 (特히 老令母樹에서 採取한 클론의 境遇) 低調한 傾向을 보였으므로, 試驗管內 挿木法을 通하여 發根率 自體의 增進을 期待하기는 어려우나 여러가지 要因操作이 容易하여 發根生理에의 應用이 可能한 것으로 思料된다.

SUMMARY

A series of rooting experiments of *P. radiata* needle fascicles were carried out in test tubes using peat-pumice medium to see a possibility of rooting fascicles in test tubes and, if possible, to find out whether that method can improve the rooting.

Typical rooting of clones from ortets aged 3, 7, 11, 18, and over 40, respectively, was 57%, 47%, 18%, 4%, and 2%. The effect of ortet ages upon rooting of fascicle cuttings was significantly exerted in these experiments. In the older ortets these results are not as good as those often obtained in a glasshouse.

Hormones tested (indolebutyric acid and abscisic acid) had no significant effect on rooting and neither did a series of fungicides tested. Needle fascicles placed in an 18 hour day at 20°/10°C (day/night temperature) rooted signficantly better than those in a 10 hour day at the same temperature regime. The latter in turn rooted better than those set under a 10 hour day at 15°/5°C. Clonal differences in rooting ability were also distinct in every trial of the present experiments with needle fascicles.

INTRODUCTION

Since the possibility of rooting needle fascicle cuttings in pines was discussed in the 1930s (Jacobs, 1939; Thimann and Delisle, 1939), needle fascicle cuttings have attracted forest tree breeders as their use would make it possible to raise a mass of isogenic

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plants for breeding or experimental purposes.

Most experiments so far with many pine species have shown that the fascicles taken from ortets younger than ten years of age root reasonably well but the ones from the older age do poorly or do not root at all (see review in Table 7). In practical tree breeding, therefore, it has been difficult to use fascicle cuttings for the vegetative propagation of selected plus trees which are older than ten years.

Another difficult problem in the fascicle cuttings is the shoot development of the rooted fascicles. Though an ordinary needle fascicle has a latent bud capable of developing a shoot, rooted fascicles do not develop shoots readily from the fascicular buds. However, this problem has been solved since Jeckalejs (1956) suggested a method to force the development of active fascicular buds by excision of distal portions of new shoots of pines at the beginning of the growth period before the fascicles were collected as cutting materials. This method has been successfully practised by Isikawa and Kusaka (1959), Hong (1974) and by Thulin and Faulds (person. comm.).

The first mention of the possibility of vegetative propagation in *P. radiata* by means of rooted fascicles was by Jacobs (1939). Subsequently Mergen and Simpson (1964) obtained 1% rooting of fascicles taken from 6 year old trees. Sievwright (1967) did not successfully root fascicles from 20 year old trees but obtained up to 80% rooting of fascicles from $1\frac{1}{2}$ year old trees. More improved rooting results were shown by Kummerow (1966, 1969) who reported 28%, 75%, 20% and 0% rooting of fascicles taken from 2, 5, 8 and 28 year old ortets, respectively. Faulds (unpublished) was obtaining up to 80% rooting from young trees, and up to 30% from trees older than 10 years as the time when these experiments started.

We have tried a series of rooting experiments with budded needle fascicles of *P. radiata* in test tubes. It was believed that by bringing the fascicles into a laboratory environment it might be possible to apply a measure of specialised treatment and environmental control which would provide better rooting than in the glasshouse, particularly for difficult clones.

This report details the effects of ortet ages, clones, hormones, fungicides and environmental conditions upon rooting of fascicle cuttings in test tubes, and compares the results with those obtained in a glasshouse.

These experiments were carried out at Forest Research Institute, Rotorua, New Zealand. The authors wish to express appreciation to Mr. T. Faulds and Mr. R. Morton for their production of budded fascicles and collection of the cutting materials.

MATERIALS AND METHODS

PREPARATION OF BUDDED FASCICLES

Needle fascicles which bear well-defined buds before they are set as cuttings develop a shoot easily after they root. In order to force the fascicle buds at the base of needles to develop, the tips of branches were nipped off about two months before the collection of fascicles from the trees. In two months the fascicular buds developed sufficiently to be used as cutting materials; with larger buds on fascicles at the top of the branch and the smaller ones lower down. Prior to setting the budded fascicles were cut at their base without the attachment of any bark, using a razor blade.

MEDIUM AND PLANTING IN TEST TUBES

Following a series of preliminary investigations a mixture of peat and pumice (4:6) was used as a cutting medium. Twenty grams of the dry medium was put into a test tubes (38×200mm) and six ml of water or hormone solution were added to each tube. Four needle fascicles were planted about 4mm deep in medium in each test tube and the mouth of tube was covered with a sheet of polythene film and rubber band tight enough to keep the humidity inside the tube.

The tubes in which fascicle cuttings were planted were placed under light shelves installed with four 40W cool-white fluorescent lamps on an area of 67cm ×133cm. The room of the light shelves was adjusted to a temperature of 22°C-27°C and a 16 hour-day length. Most fascicle rooting experiments were carried out in the light-shelf room, the major exception being the growth cabinet experiments (Trial 6).

RESULTS

TRIAL 1. Effects of ortet ages on fascicle rooting

Five different age groups of trees: 3, 7, 11, 18 and over 40 years old were used as materials. Five clones were included in each age group and forty needles of each clone were set in the tubes in October, 1974 being 1000 fascicles in total.

Callus was formed well on the cut surface of the fascicles in two weeks and some fascicles rooted within a month. Rooting results assessed six months after settingare shown in Table 1.

As expected, the rooting of fascicle cuttings decreased with increased age of the ortets, showing average rooting 57%, 47%, 18%, 4% and 2%, respectively, with fascicles from ortets of ages of 3, 7, 11, 18 and over 40. Within the same age group, clonal difference in rooting ability was very distinct; the difference ranged from 30 to 90% in 3 year old clones, 15 to 73% in 7 year old ones, 3 to 55% in 11 year old ones, 0 to 10% in 18 year old ones and 0 to 3% in over 40 year old ones.

Table 1. Rooting of fascicles taken from different age groups of ortets. Experimental period; 25 October 1974-29 April 1975.

(rooting %)

Ortet ages	Clones					
3	3-1	3-2	3-3	3-4	3-5	mean
	85.0	42.5	30.0	90.0	37.5	57%(61)*
	871 series					
7	078	079	084	087	098	mean
	55.0	15.0	37.5	72.5	52.5	47%(53)
	266 series					
11	470	475	477	492	496	mean
	2.5	55.0	15.0	7.5	7.5	18%(43)
	268 series					
18	186	206	217	219	224	mean
	0	7.5	10.0	2.5	0	4% (10)
	uninodal					
Over 40	501	502	503	504	505	mean
	2.5	2.5	0	0	2.5	2% (13)

*The numbers in parentheses show rooting percentages for fascicles of the same clones set in the same clones set in the glasshouse by T. Faulds in the same season.

In the 871 series (7 year old) Clone 087 was the best rooter and Clone 079 the poorest. In the 266 series (11 year old) the best rooter was Clone 475 and the poorest Clone 470. Two hundred and sixty-eight series clones (18 year old) were infected by needle disease and gave poorer results of rooting.

In the younger age classes rooting in the test tubes was comparable to that achieved in the glasshouse by T. Faulds in a parallel experiment. With ortet ages over 10, however, rooting in the test tubes was poorer than in the glasshouse.

TRIAL 2. Effects of IBA-ABA on rooting

Fascicles from five clones of the 266 series (ortet age 11) were set in 25 different media comprising combinations of IBA (indolebutyric acid) and ABA (abscisic acid) in October, 1974. The 25 treatments were combinations of five different concentrations: 0, 5, 10, 25 and 50 ppm of IBA and ABA, respectively. Each treatment had 3 ml of IBA and 3 ml of ABA added to 20 grams of the soil medium in one test tube, and was replicated five times using 500 fascicles in total.

From the rooting results 6 months after setting (Table 2), no significant treatment effects were found upon rooting of fascicles but the clonal effect was again clear-cut. The best rooting resulted from Clones 460 and 475 and the poorest from Clone 470 as observed in the previous trial.

TRIAL 3. Effects of locations on rooting

In this trial effects of three different locations on rooting were examined. The three locations were as follows:

(1) Glasshouse — where cuttings are set in a frame covered with polyethylene film,

(2) Light shelves — as described in "Medium and planting in test tubes",

(3) Shade — a shade frame made with 10 mesh insect net.

Sixty fascicles were collected from each of ten clones of the 268 series (ortet age 18) and 200 fa-

Table 2. The effects of indolebutyric acid and abscisic acid on rooting of fascicles.
Experimental period; 4 October 1974-27 February 1975.

(rooting %)

ABA	IBA	266 series clones 445	456	460	470	475	Mean
0 ppm	0 ppm	25	25	75	0	25	30
	5	50	0	50	0	50	30
	10	50	25	50	25	25	35
	25	0	0	50	0	50	20
	50	50	50	75	0	75	50
5	0	25	25	25	0	0	15
	5	50	0	25	0	0	15
	10	25	0	25	0	25	15
	25	25	25	0	0	50	10
	50	25	0	75	0	50	30
10	0	25	50	25	25	25	30
	5	25	0	25	0	50	20
	10	0	0	0	0	75	15
	25	0	0	50	25	50	25
	50	25	50	75	0	50	40
25	0	25	75	100	0	50	50
	5	25	0	50	25	75	35
	10	50	25	50	0	25	30
	25	0	50	0	25	25	20
	50	25	0	50	50	25	30
50	0	25	75	75	0	0	35
	5	25	0	0	25	25	15
	10	0	0	75	0	50	25
	25	75	50	25	25	75	50
	50	50	50	75	0	0	35
Mean		28	23	45	9	38	

Treat.	ABA (mean)	IBA (mean)
0 ppm	33%	32%
5	17	23
10	26	24
25	33	25
50	32	37

Table 3. Rooting of fascicles in three different locations.
Experimental period; 16 October 1974-26 February 1975.

(rooting %)

Location	268 series clones 165	174	186	217	236	252	189	215	204	234	Mean
Glasshouse	0	0	0	0	5	0	10	15	0	5	3.5
Light shelves	0	0	0	0	0	0	5	0	0	0	0.5
Shade	0	0	0	0	0	0	0	0	0	0	0

scicles (20 per clone) set in test tubes were placed in each of the three locations in October, 1974.

As shown in Table 3, the fascicles placed in the glasshouse rooted slightly better than those in light shelves but there was no significant difference. No fascicles rooted in the shade and rooting overall was poor.

TRIAL 4. Effect of CO_2 on rooting

In order to see the effect of CO_2 (carbon dioxide) on rooting, the gas (made from barium carbonate and sulphuric acid) was injected weekly into the fascicle tubes for five successive weeks commencing two weeks after setting on 15 October, 1974. Fifty-six fascicles taken from each of five clones of the 268 series (280 fascicles in total) were set in 70 test tubes. One half of them was injected with CO_2 (to a concentration in the tube at 0.5%) and the other half was used as the control.

Four and a half months after setting, two fascicles out of 140 CO_2 treated fascicles rooted, but none from the control (Table 4-1).

In another experiment the effect of trapping CO_2 in the tube on rooting was examined in May, 1974. Once fascicles formed callus tissue (two weeks after setting) the CO_2 in the air inside the tube was trapped with sodium hydroxide (NaOH) pellets kept in a vial inside the tube without touching fascicles. The fascicles were from Clone 870-011 (ortet age 7) and 20 fascicles were set in both the NaOH treatment and the control.

Trapping of CO_2 in the tube slowed down or inhibited considerably rooting, with treated fascicles showing 45% rooting compared with 80% rooting of the control two months after setting (Table 4-2).

Table 4-1. The effect of CO_2 on rooting of fascicles. Experimental period; 15 October 1974-25 February 1975.

(rooting %)

Treatment	268 series clones					
	165	174	234	236	252	Mean
$+CO_2$	7.2	0	0	0	7.2	2.9
Control	0	0	0	0	0	0

Table 4-2. The effect of trapping CO_2 on rooting fascicles. Experimental period; 29 May 1974-6 September 1974.

(rooting %)

Treatment	Clone 870-011	
	2 months after setting	3 months after setting
$-CO_2$	45	65
Control	80	85

TRIAL 5. Effects of fungicides on rooting

The effects of some fungicides on the rooting of fascicles were examined. Six fungicide treatments used were as follows:

(1) Spray with 1×10^6 unit penicillin solution
(2) Soak in 1×10^6 unit penicillin solution
(3) Soak in 250 ppm Benlate solution
(4) Dip in 10% Captan powder
(5) Spray with 500 ppm chloramphenicol solution
(6) Control

Five uninodal clones (ortet age 40) were used for this experiment. Twenty fascicles (4 from each clone) were included in each treatment and 120 fascicles in total were set in October, 1974.

The results, assessed four and a half months after setting, showed no better rooting from the fungicide treatments than the control as shown in Table 5.

Table 5. The effect of fungicides on rooting. Experimental period; 10 October 1974-26 February 1975.

(rooting %)

Treatment	uninodal clones					
	506	513	514	517	519	Mean
Benlate	0	25	0	25	0	10
Captan	0	0	0	0	0	0
Penicillin soak	0	0	0	0	50	10
〃 spray	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0
Control	0	0	0	50	0	10

TRIAL 6. Effects of temperature and length of day on rooting

In this experiment the effects of four treatments, each with subtreatments were examined on rooting. The treatments are as follows:

(1) Growth cabinet conditions:

Cabinet 1- 18 hour day, 6 hour night at 20°/10°C

Table 6. Effects of temperature and length of day on rooting of fascicles.
Experimental period; 4 March 1975-6 August 1975.

(rooting %)

Treatment	266 series 456	477	492	445	460	Mean	
20°C/10°C, Top, IBA, Wet	8.3	5.0	4.2	0	0	3.5	
18 hrs Dry	8.3	5.0	4.2	0	0	3.5	
Cont, Wet	12.5	4.6	0	0	0	3.4	
Dry	8.3	0	0	0	0	1.7	
Bottom, IBA, Wet	4.2	15.0	0	0	0	3.8	
Dry	8.3	10.0	4.2	0	0	4.5	
Cont, Wet	8.3	0	4.2	4.2	0	3.5	
Dry	25.0	0	0	0	0	5.0	
							3.6*
20°C/10°C, Top, IBA, Wet	0	0	0	0	0	0	
10 hrs Dry	0	4.2	0	0	0	0.8	
Cont, Wet	0	0	0	0	0	0	
Dry	0	9.1	0	0	0	1.8	
Bottom, IBA, Wet	0	0	8.3	0	0	1.7	
Dry	8.3	0	0	0	0	1.7	
Cont, Wet	0	0	0	0	0	0	
Dry	4.2	0	0	0	0	0.8	
							0.9*
15°C/5°C, Top, IBA, Wet	0	0	0	0	0	0	
10 hrs Dry	0	0	0	0	0	0	
Cont, Wet	0	0	0	0	0	0	
Dry	0	0	0	0	0	0	
Bottom, IBA, Wet	0	0	0	0	0	0	
Dry	0	0	0	0	0	0	
Cont, Wet	0	0	0	0	0	0	
Dry	0	0	0	0	0	0	
							0*
MEAN	6.0	3.3	1.6	0.3	0		

* Average rooting percentages in each growth cabinet

(day/night temperature)

Cabinet 2- 10 hour day, 14 hour night at 20°/10°C (day/night temperature)

Cabinet 3- 10 hour day, 14 hour night at 15°/5°C (day/night temperature)

(2) Position of needle fascicles:

Top—fascicles taken from the top half of the portion of the branch which developed budded fascicles

Bottom—fascicles from the bottom of the portion of the branch which developed fascicles.

(3) Hormone:

IBA—fascicles soaked in 20 ppm IBA solution for 24 hours before planting.

Control—fascicles soaked in water for 24 hours before planting.

(4) Moisture of medium:

Wet- 20 grams medium with 9ml of water

Dry- 20 grams medium with 6ml of water.

Therefore, 24 treatments in total were applied to

the fascicles of five clones of the 266 series (ortet age 11). Twenty-four fascicles/treatment/clone were used and 2880 fascicles in total were set in early March, 1975.

Rooting of fascicles assessed five months after setting is shown in Table 6. The effects of the three different growth cabinet conditions on rooting were clear. Fascicles set in the 18 hour day at 20°/10°C showed higher rooting (3.6%) than those set in the 10 hour day at the same temperature regime (0.9%). No rooting was obtained from the fascicles set in the cabinet of 10 hour day at 15°/5°C regime. This treatment effect was shown to be highly significant by analysis of variance.

The effects of the other treatments: position, hormone, and moisture upon rooting were negligible. Clonal difference in rooting ability was distinctive also in this trial. Clone 460 did not root at all in any condition but Clone 456 rooted up to 25%.

DISCUSSION

A considerable range of tissue culture media were tested prior to setting on the peat/pumice mixture used in these experiments, and differing media have also been tested unsuccessfully by Giles (DSIR, Palmerston North, NZ, person. comm.). The use of agar for rooting fascicle cuttings does not seem possible, the result being heavy fungal contamination. Thus the treatments ultimately tested did not differ too greatly from those used conventionally in the glasshouse.

In general callus was formed well in two weeks on the cut surface of the fascicles with the peat/pumice mixture set in test tubes and some fascicles rooted within a month. The present experiments have shown a possibility of rooting of fascicle cuttings in test tubes and the rooting percentages are considerably higher than those reported in the liturature for *P. radiata* and other species (Table 7). But the rooting was a little lower in tubes than that obtained with the same clones in the glasshouse (Faulds, unpublished), particularly when older ortets are considered.

The problem of juvenility and maturity of ortets occurred also with the needle fascicle cuttings of *P. radiata*. The rooting of fascicle cuttings decreased with increased age of ortets in this experiment as previously reported (Toda 1948, Kummerow 1969; Larsen and Dingle 1969). It is noteworthy to see a sudden drop in rootability of fascicles after a certain age of ortets. Such drop in rooting has been thought to be due to the phase change from the juvenile to the mature. The phase change seems to appear at the age of around 10 to 15 in *P. radiata*, whereas it does earlier than that in other pine species. The fact suggests that *P. radiata* holds juvenility comparatively longer than the other species.

The reason why test tube culture with materials from older ortets proved less successful than Faulds standard glasshouse method is uncertain and is perhaps not important to the future programme with fascicles. The points emerging from the report which may be of value to the fascicle programme are (i) the illustration of the importance of temperature already shown for stem cuttings by Cameron and Rook (1974) and (ii) the illustration that rooting was increased by a longer day/shorter night. It should be noted that this latter was probably not a simple photoperiod effect, but rather an effect of having a longer day with a warm temperature and high light intensity. A third point demonstrated of importance was the necessity for some level of atmospheric CO_2 for successful rooting—a not unexpected finding, but one relevant to the use of sealed systems with high humidity.

As with almost previous experience with *P. radiata* cuttings, hormones again proved no significant effect on rooting and neither did a series of fungicides tested. Clonal effect on rooting was very distinct with fascicle cuttings in these experiments.

The prospect that it might be possible to improve on rooting by moving to a system mid-way between conventional vegetative propagation and tissue culture has not in fact been realized in the results of the experiments reported here. However, the rooting of fascicles in test tubes shown in these experiments seems to be a useful method for physiological study of rooting in pine species because of its advantages in manipulating environmental factors and in periodical observation of rooting process.

Table 7. Review on the rooting experiments of needle fascicle cuttings in pines.

Species	Age of ortets	Rooting %	Authors
P. banksiana	2 years	15-70	Rudolph and Nienstaedt (1964)
〃	5	5-10	〃 〃
P. contorta	2	20	Larsen and Dingle (1969)
〃	4	4	〃 〃
〃	6	1	〃 〃
P. densiflora	1	35-68	Toda (1948)
〃	3-7	3-15	〃
〃	13	0	〃
〃	6	3-47	Toda (1952)
P. densi-thunbergii	2	43	Toda (1948)
P. echinata	1 1/2	2-18	Zak and McAlpine (1957)
〃	2-32	0	Hare (1965)
P. elliottii	2	42-58	Zak and McAlpine (1957)
〃	3	16-19	Reines and Bamping (1964)
〃	3	1	Mergen and Simpson (1964)
〃	4	0	〃 〃
〃	4-21	0	Hare (1965)
P. glabra	4	3-15	〃
〃	5	0	〃
〃	6-10	1	〃
P. monticola	2	48-59	McDonald and Hoff (1970)
P. palustris	3-31	0	Hare (1965)
P. radiata	1 1/2	40-80	Sievwright (1967)
〃	1-3	28	Kummerow (1966)
〃	6	1	Mergen and Simpson (1964)
〃	7-9	20	Kummerow (1966)
〃	28	0	〃
〃	5	62-88	Kummerow (1969)
P. resinosa	2	32-68	Jeckalejs (1956)
P. rigida	3	3	Yim (1962)
〃	10	5	〃
P. strobus	2	8-74	Thimann and Delisle (1942)
P. taeda	3	24-51	Reines and Bamping (1964)
〃	4	0	Mergen and Simpson (1964)
〃	2-25	0	Hare (1965)
P. thunbergii	12	0-54*	Isikawa and Kusaka (1959)

*7 fascicles rooted out of 13 taken from only one tree.

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