Auxins and Gibberellin-like Substances Existing in the Shoots of Conifers and Their Roles in Flower Bud Formation and Flower Sex Differentiation

By

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I. Introduction

Naturally occurring growth substatances in higher plants can be broadly classified into four groups by the difference of physiological action : auxin, gibberellin, cytokinin and inhibitor. The auxin is a phytohormone which is first descovered in higher plants, and it is a general term for the substances showing physiological action resembling indoleacetic acid. At the present time, however, it is known that indoleacetic acid and the like compounds are not the only naturally occurring auxins in higher plants, and that in some plants non-indole compounds also exist as an important natural auxin. The gibberellin also is an important growth substance in higher plants. Up to the present twenty-three kinds of gibberellins are isolated from higher plants and fungi, and their chemical structure is determined. However, a noteworthy fact is that helminthosporol having different chemical structure from gibberellin but showing gibberellin-like action was recently isolated from a fungus, *Helminthosporium sativum*. Although the cytokinin is a relative late comer in the isolation from plant tissues, recently zeatin was isolated from maize kernels. Furthermore, there are many indirect evidences that the cytokinin exists in higher plants. In higher plants, various inhibitors are present. Recently, abscisic acid was isolated from cotton fruits. This substance, thereafter, is found in several plants, and its physiological action attracts the notice of investigators. In addition, the possibility remains that phytohormones different from the above four groups may be isolated from higher plants in the future. Although these phytohormones are distributed widely in the vegetative kingdom, their kind or amount seems to differ with the kind of plants.

As for studies on growth substances present in conifers, bygone studies were performed mainly on auxin, especially ether-soluble auxin. Studies on ether-insoluble auxin, gibberellin, etc. are very few in number. As the growth and differentiation of the plant, however, are regulated by the interaction of various growth substances, it is most necessary to analyse not noly ether-soluble auxin but also other growth substances such as ether-insoluble auxin, gibberellin, cytokinin and inhibitor, for elucidating physiological phenomena in conifers. Recently, it has been reported even that in a certain plant, indole auxins do not always play the leading role in the growth and differentiation of the plant.

Gibberellin plays an active role in flower formation in many cold-requiring and long-day plants. In forest trees, applied gibberellin induced the flower bud formation of many

species belonging to *Taxodiaceae* and *Cupressaceae*, but not that of *Pinaceae* and *Cunninghamia* species. Such a difference of the response of the tree species to growth substances has been recognized on the action of auxin to the rooting of cuttings. The author[1~2] repoted in previous papers that gibberellin promoted the flower bud formation of *Cryptomeria japonica*, and that growth substances in new shoots of the plant changed considerably in relation to flower induction by spraying with gibberellin. Saito[3] reported that the sex differentiation of *Pinus densiflora* and *P. thunbergii* could be controlled by pinching the shoot or spraying with naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid over the shoot. The author[1~5] also was successful in causing sex transiton from male strobiles of *Cr. japonica* to female by pinching and spraying with gibberellin. From these experimental results, it is suggested that growth substances may play an important role in flower bud formation and flower sex differentiation in conifers.

The present investigations were undertaken to ascertain growth substances existing in conifers for the purpose of explaining physiologically the differences in the response of tree species to flower induction by gibberellin, and to make clear the relation between flower bud formation or flower sex differentiation and endogenous growth substances in conifers.

Ⅱ. Auxins and gibberellin-like substances present in the shoots of conifers

1. Materials and methods

The following nine species growing in the nursery of the author's university were examined to ascrtain the presence of auxins and gibberellin-like substances: *Pinus densiflora, P. thunbergii, P. elliottii, P. taeda, P. strobus, Cryptomeria japonica, Metasequoia glyptostroboides, Cunninghamia lanceolata* and *Chamaecyparis obtusa*. As to the material for extracting the growth substances, growing shoots were used. The samples, as shown in Tables 1 and 2, were collected from comparatively young trees of 3~20 years old in a growth period between March and July.

Extraction and separation of auxin:

The method of extraction and separation of auxin from shoots of conifers is shown in Fig. 1.

20 g shoots in fresh weight were homogenized in a blender and extracted with three changes of 70 ml of 80 % methanol for a total period of 24 hours at 0~2°C. The methanol extract was filtered, and concentrated in a rotary evaporator at 50°C under reduced pressure. The resulting residue was taken up in water at room temperature, and filtered. The filtrate was adjusted to a pH of 8.5 with 5 % Na_2CO_3 and shaken four times with preoxide free ether to yield the neutral-ether fraction. The remaining filtrate was next adjusted to a pH of 2.9 with 0.5 N HCl and shaken four times with ether to yield the acid-ether fraction. The ether fractions were washed with water, dehydrated with anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced

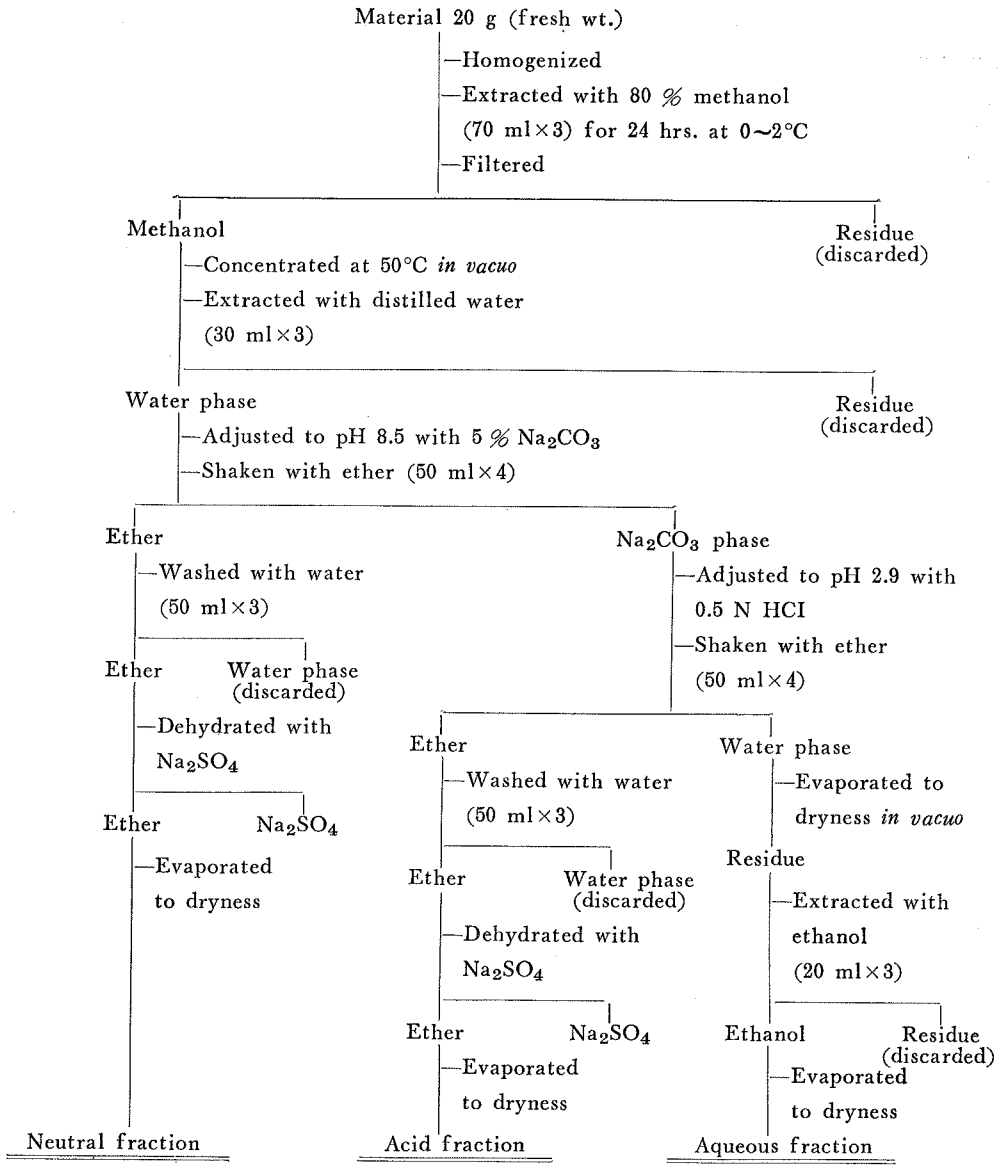

Fig. 1. Flow diagram showing procedure for extraction and separation of auxins from the shoots of conifers.

pressure. Srivastava[6] had reported that, if the ether fractions were washed with water and dried over anhydrous Na_2SO_4 prior to paper chromatography the contamiants like sugars or amino acids were not detected on the chromatograms. The water phase left after ether extracion was evaporated to dryness under reduced pressure and the residue was dissolved in absolute ethanol. The ethanol extract was filtered and evaporated to dryness. This fraction, containing ether insoluble substances, was termed the aqueous fraction.

All fractions were taken up in a small volume of ethyl acetate or ethanol for paper chromatographic studies and streaked on Tōyō No.50 filter paper (20 × 40 cm). Chromatograms were developed at about 25°C in the dark in glass cylinders by a ascending solvent system containing *iso*-propanol, ammonium hydroxide, and water (8 : 1 : 1,v/v/v), and they were removed when the solvent front reached about 25 cm from the starting-line. Indoleacetic acid (IAA) and Indoleacetonitrile (IAN) were run in parallel with plant extracts in order to determine their standard position. The chromatograms after development were dried, and used for bioassay and color reaction test.

Extraction and separation of gibberellin-like substances:

Shoots (40～50 g fresh weight) were ground, and extracted with three changes of 100 ml of 80 % methnol for a total period of 24 hours at room temperature. The methanol extract was then filtered and the filtrate was evaporated to dryness under reduced pressure. The resulting residue was dissolved in 100 ml of distilled water. The water phase was filtered, and evaporated to dryness under reduced pressure. The resulting residue was dissolved in a small volume of ethanol for paper chromatography.

The ethanol extract was streaked on Tōyō No.50 chromatographic paper and developed by the ascending method with a mixture of *iso*-butanol, methanol and water (80 : 5 : 15, v/v/v) at about 25°C in the dark, until the solvent reached about 25 cm from the origin. In order to separate gibberellin-like substances from inhibitor β which checks the action of gibberellin on rice seedlings, the developed chromatograms were divided into two parts corresponding to Rf 0～0.5 and Rf 0.5～1.0, and each part was cut into small segments and eluted with methanol. The eluate from Rf 0～0.5 contained the greater part of gibberellin-like substances, and that from Rf 0.5～1.0 the inhibitor β. The former was termed the fraction Ⅰ, and the latter the fraction Ⅱ, respectively. Each fraction was dried, taken up into a small amount of ethanol, and rechromatographed with *iso*-propanol, ammonium hydroxide and water (8 : 1 : 1,v/v/v) as described above.

Bioassay of auxin:

The chromatogram strip equivalent to the extracts of 5 g shoots in fresh weight, except the aqueous fractions of *P. densiflora*, *P. thunbergii* and *P. strobus*, was cut off along the solvent flow. The strip was again divided into ten equal segments at right angles to the direction of the solvent flow. Each segment was then placed in glass tubes 3 cm in diameter and 5 cm in height, and 1.5 ml of phosphate-citrate buffer of pH 5.0 containing 2 % sucrose was put into each tube. The auxin activity was measured by the author's pine hypocotyl test[7] using *P. thunbergii*. When the hypocotys had reached 2.0 to 2.5 cm in length, sections of 4 mm length were cut off at about 2 mm below the tip, and ten secions were put into the test solution of each tube after being presoaked for one hour in distilled water. The tubes were then allowed to stand in the dark at 25°C, and after 30 hours the length of each section was measured under a binocular with ocular micrometer. All manipulations with the hypocotyl sections were performed in the dark or under a red safe-light. Under these conditions, the response of pine hypocotyl sections to auxin

is shown in Fig. 2. The minimum concentration of IAA detectable in this bioassay is approximately 0.005 mg/l. Their response to gibberellin is very small, except when auxin exists together.

Bioassay of gibberellin:

Gibberellins were measured by the rice seedling method as described by Murakami[8] and Ogawa.[9] A dwarf variety "Tamanishiki" and a normal variety "Nōrin No.22" were used as the test plants. The developed chromatograms were dried and cut tsransversely into 10 equal strips. Each strip was placed in 3×12 cm glass tubes containing 2.0 to 2.5 ml of distilled water. Seven rice seedlings, whose coleoptiles attained about 1 mm, were planted in each tube. The tube was sealed with a sheet of polyethylene film to prevent drying, and allowed to stand in a glass incubator kept at 30°C under continuous light condition, supplied by illuminating fluorescent lamp at night. After 7 days, the length of the second leaf sheath was measured. A typical growth response of the second leaf sheath of rice seedlings to gibberellin A_3 is shown in Fig. 3.

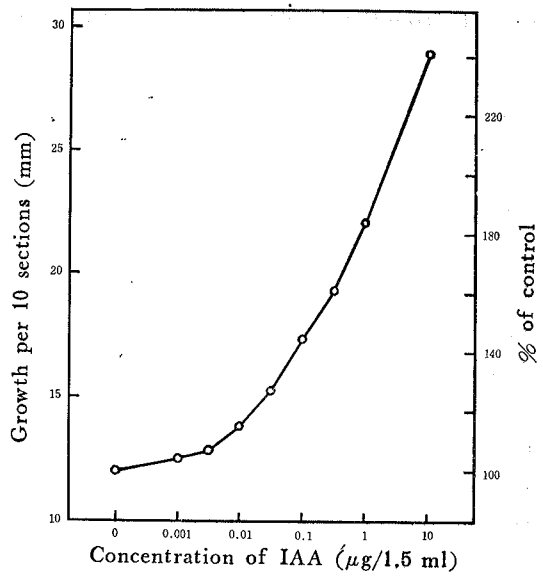

Fig. 2. Response of pine hypocotyl section to indoleacetic acid.

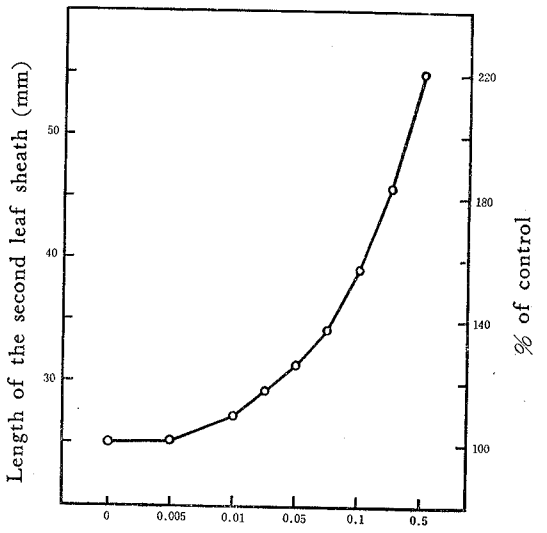

Fig. 3. Response of the second leaf sheath of rice seedling (a dwarf variety "Tamanishiki") to gibberellin A_3.

Color test of auxin:

For the identification of various compounds, the dried chromatograms after development were sprayed with a modified Salkowski reagent (0.05 M $FeCl_3$ in 35 % $HClO_4$), the Ehrlich reagent (2 % p-dimethylaminobenzaldehyde in 80 % alcohol-20 % concentrated HCl), and the ninhydrin reagent (2 % ninhydrin in water-saturated butanol).

2. Results

1) Auxins in the shoots of conifers

Figures 4 to 6 show the results of the auxin bioassay on the chromatograms of the

extracts from the shoots of various conifers using pine hypocotyl sections. Auxin activity was represented in percentage to the elongation of controls without plant extracts.

In the neutral fraction, as shown in Fig. 4, several zones showing auxin activity were recognized on every chromatogram of all species examined, though they were not especially remarkable. No growth inhibiting zones were detected in the neutral fraction. Promoting substances in the neutral fraction seem to differ fairly among tree species. In *P. densiflora* a promoting zone was found at Rf 0.5~0.8, while at least four promoting zones were detected at Rfs 0~0.1, 0.3~0.5, 0.6~0.7 and 0.8~1.0 in *P. thunbergii* and *P. strobus*. There were two promoting zones at Rfs 0.5~0.7 and 0.8~1.0 in *P. elliottii* and *P. taeda*. A noticeable promoting zone was found at Rf 0.1~0.2 in *Cr. japonica*, and at Rf 0.9~1.0 in *M. glyptostroboides*. Two weak promoting zones were also detected at Rfs 0.3~0.5 and 0.8~0.9 in *Cu. lanceolata*, and at Rfs 0.2~0.3 and 0.7~0.9 in *Ch. obtusa*, respectively. Among these promoting zones, a zone of Rf 0.6~0.7 was especially conspicuous in *Pinus* species. A zone of Rf 0.8~1.0, which was seen in four pines, *Metasequoia*, and *Chamaecyparis*, corresponded to the position of IAN developed at the same time. In the neutral fraction, no colored spots could be detected on the chromatograms of all species examined, when sprayed with both Ehrlich's and Salkowski's reagents.

In the acid fraction (Fig. 5), two specially remarkable growth-promoting zones were detected at Rf 0.4~0.5 and Rf 0.6~0.7 on the chromatograms of the extracts from *Pinus* species. One of them, located at Rf 0.4~0.5, was also found in *Cr. japonica*, *M. glyptostroboides*, *Cu. lanceolata* and *Ch. obtusa*. It corresponded to the position of IAA in the guide chromatogram, being provisionally termed "Factor Ⅰ". The other promoting zone found at Rf 0.6~0.7 gave higher activity in *Pinus* species, especially in *P. taeda* and *P. elliottii*, and it is was termed "Factor Ⅱ". This Rf value was very similar to that of indolebutylic acid (IBA) or gibberellin A, but it will not be gibberellin A because gibberellins are inactive in this bioassay. Probably the growth promotion of the Factor Ⅱ is considered to be due to the presence of auxins like IBA. On the chromatograms of *Pinus*, *Cryptomeria*, and *Metasequoia*, moreover, weak promoting zones were observed near the starting line or the solvent front. Hardly any growth inhibiting zones were detected in any tree species examined. On the chromatograms of the acid fraction, except for those of *P. elliottii* and *Cu. lanceolata*, color spots could be detected at Rfs around 0.16, 0.25 and 0.72 with Ehrlich and Salkowski reagents. All the spots, however, were indistinct and unstable, and their Rf values did not correspond with those of two remarkable growth-promoting zones, Factors Ⅰ and Ⅱ.

Fig. 6 shows auxin activity in the aqueous fraction. Although the active substances caused some variations in both the Rf value and the tailing on the chromatograms because of the presence of large quantities of impurity, a zone with very conspicuous growth-promoting activity was observed at Rf 0.2~0.5 with a maximum peak at Rf 0.3~0.4 or 0.4~0.5, on the chromatograms of all species. The zone was termed "Factor Ⅲ" in this paper. On every chromatogram of the aqueous fraction, except for that of *Ch. obtusa*, other small auxin activities were recognized at Rf 0~0.2, Rf 0.7~0.8 or Rf 0.8~1.0. On the

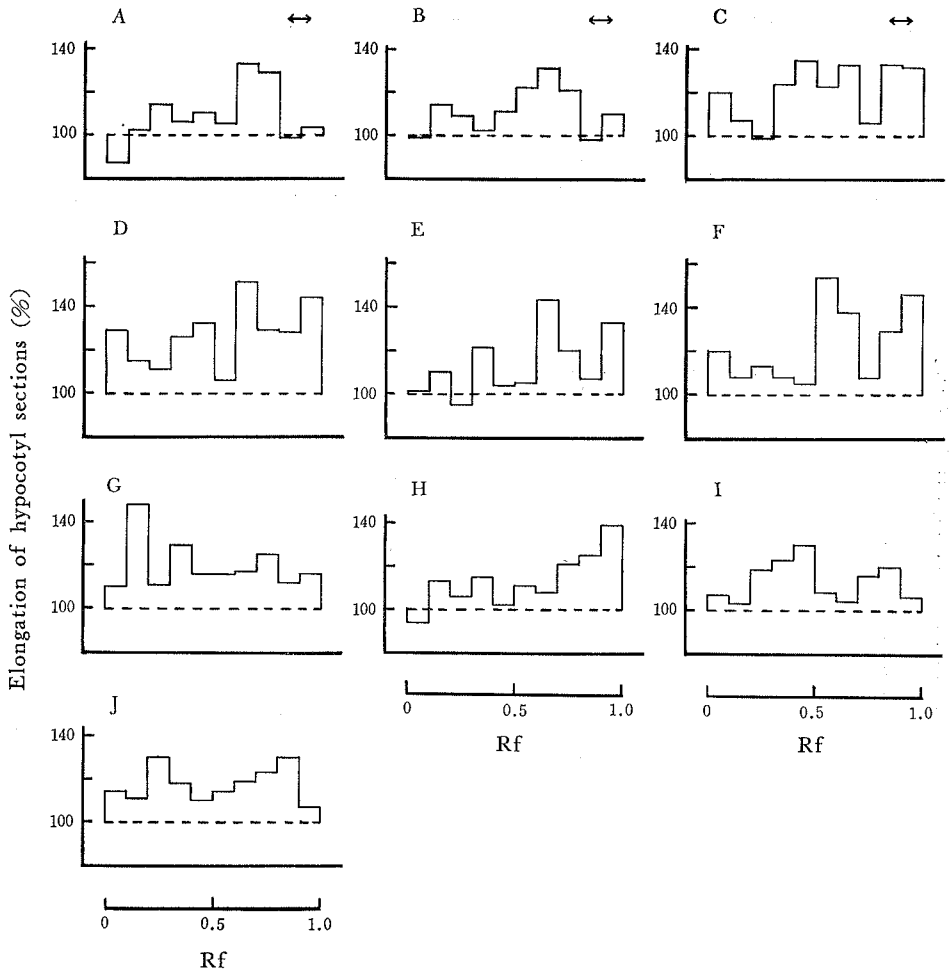

Fig. 4. Histograms showing auxin activity in the neutral fraction of extracts from the shoots of conifers. In each species, the extracts were developed with ammoniacal *iso*-propanol, and the chromatogram strip correspoding to the extracts of 5 g of shoots was assayed by the pine hypocotyl test. Broken lines denote the elongation of controls. Arrows at the top of the histograms indicate the position of IAN (Rf=0.89).

A, *P. densiflora* (March 10); B, *P. densiflora* (April 30); C, *P. thunbergii* (May 31); D, *P. strobus* (May 31); E, *P. elliottii* (June 21); F, *P.taeda* (June 22); G, *Cr. japonica* (June 3); H, *M. glyptostroboides* (June 6); I, *Cu. lanceolata* (June 28); J, *Ch. obtusa* (June 25).

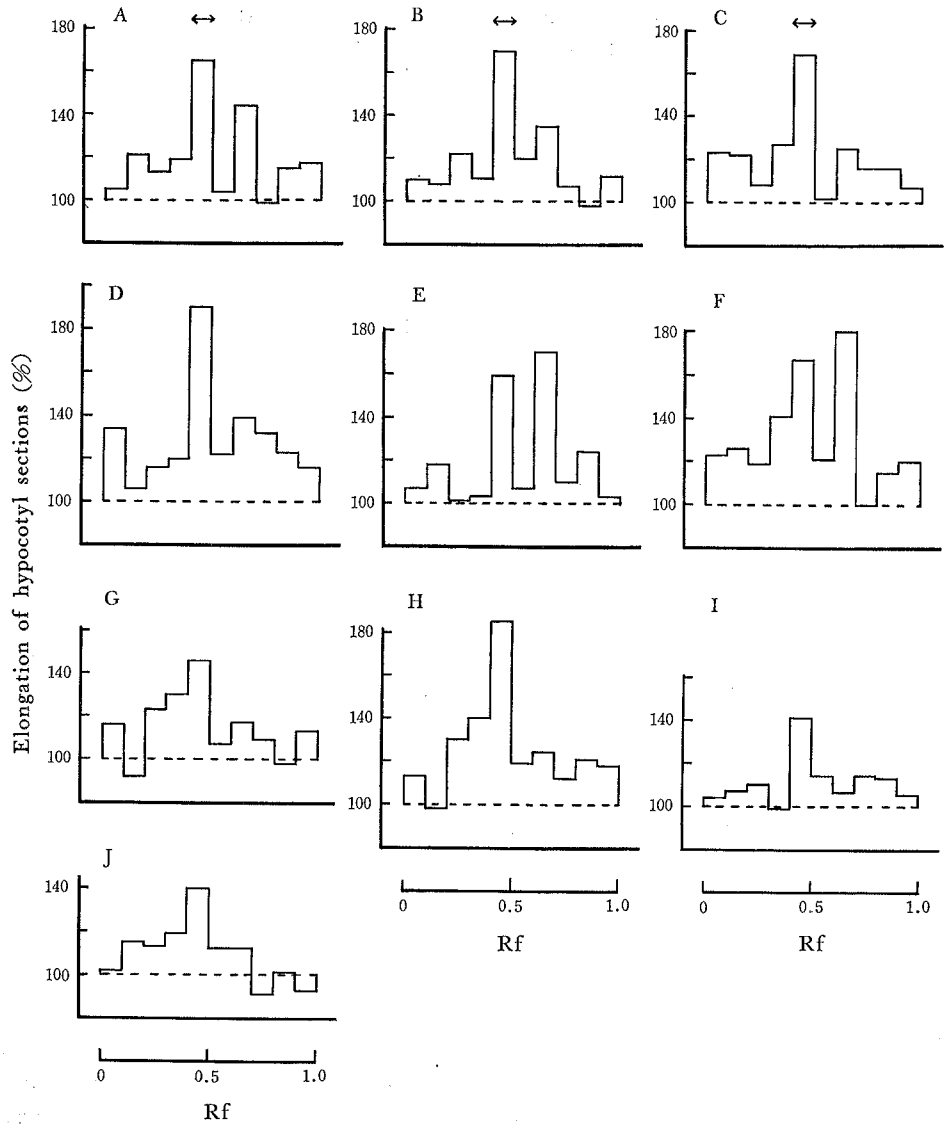

Fig. 5. Histograms showing auxin activity in the acid fraction of extracts from the shoots of conifers. Arrows at the top of the histograms indicate the position of IAA (Rf=0.45). Other descriptions are the same as those in Fig. 4.

A and B, *P. densiflora*; C, *P. thunbergii*; D, *P. strobus*; E, *P. elliottii*; F, *P. taeda*; G, *Cr. japonica*; H, *M. glyptostroboides*; I, *Cu. lanceolata*; J, *Ch. obtusa*.

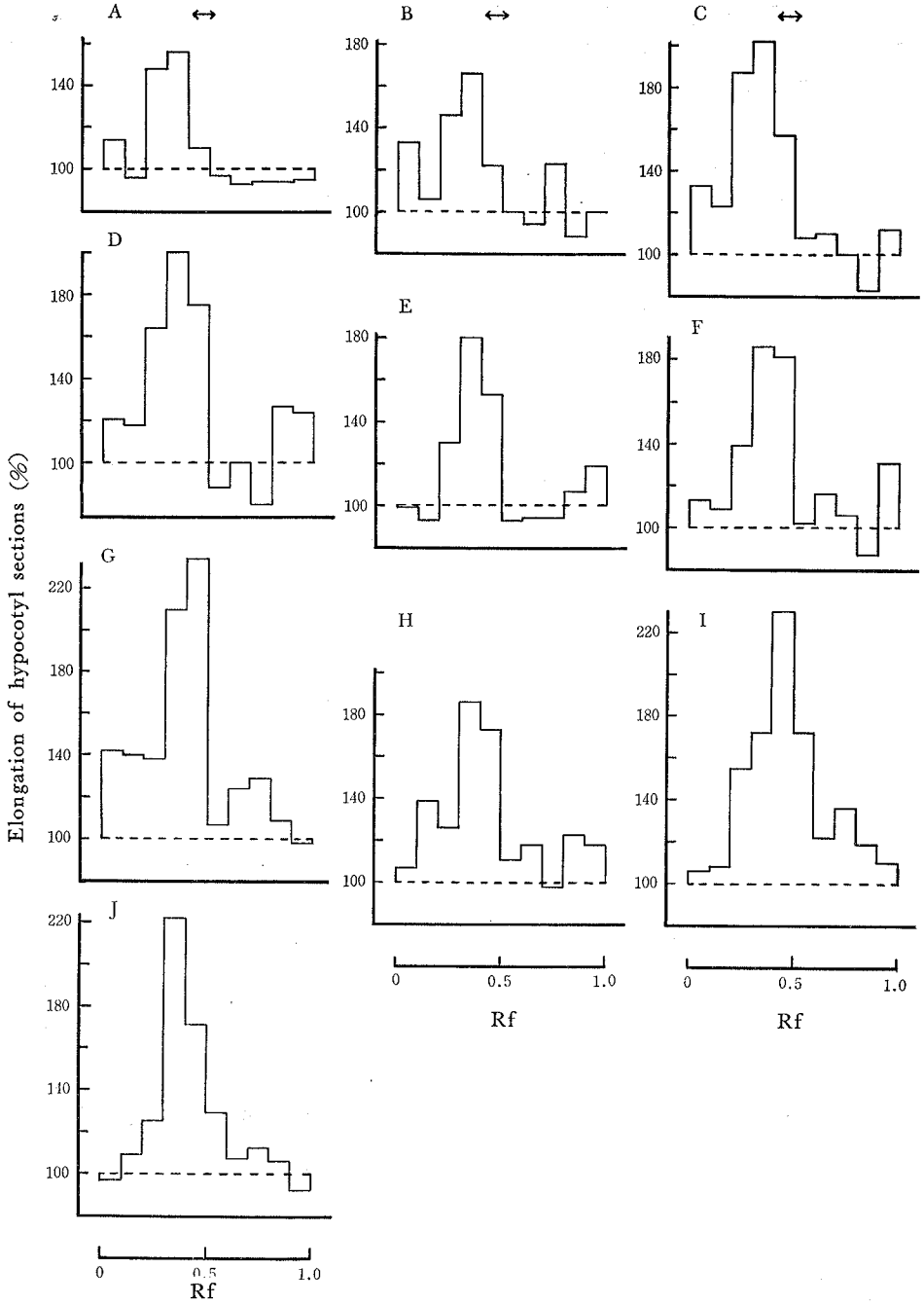

Fig. 6. Histograms showing auxin activity in the aqueous fraction of extracts from the shoots of conifers. Arrows at the top of the histograms indicate the position of IAA (Rf= 0.47). In *P. densiflora*, *P. thunbergii* and *P. strobus*, extracts from 2.5 g of shoots were used for bioassay. Other descriptions are the same as those in Fig. 4.

A and B, *P. densiflora*; C, *P. thunbergii*; D, *P. strobus*; E, *P. elliottii*; F, *P. taeda*; G, *Cr. japonica*; H, *M. glyptostroboides*; I, *Cu. lanceolata*; J, *Ch. obtusa*.

chromatograms of the aqueous fraction of a large number of species tested, color spots were found at Rf around 0.42 with Ehrlich's reagent and at Rfs around 0.42 and 0.60 with Salkowski's reagent. Amino acids were detected at Rf 0~0.28, Rf around 0.44 and Rf around 0.53 with ninhydrin reagent. Among these, an Ehrlich- and Salkowski-positive spot of Rf 0.42 and a ninhydrin-positive spot of Rf 0.44 were located in the zone of Factor Ⅲ. However, the size or position of the spots did not always correspond to the width or maximum peak of the Factor Ⅲ. These Ehrlich- and Salkowski-positive spots were also indistinct and unstable.

The approximate concentrations of auxins contained in the shoots of conifers were determined by comparison with a known quantity of IAA. The auxin concentrations estimated from Fig. 2 are listed in Table 1.

Table 1. Auxin concentrations found in the shoots of conifers.

Tree species	Tree age	Date collected	Approximate concentration (μg. IAA equivalents/100 g.f.w.)								
			Neutral auxin	Acid auxin				Aqueous auxin			Total auxin
				Factor Ⅰ	Factor Ⅱ	Others	Total	Factor Ⅲ	Others	Total	
P. densiflora	5 & 8	March 10	3.0	11.2	2.0	1.8	15.0	22.7	0.4	23.1	41.1
		April 30	3.0	14.0	1.4	1.9	17.3	30.4	4.4	34.8	55.1
P. thunbergii	5	May 31	7.8	13.6	0.8	3.0	17.4	229.3	5.2	234.5	259.7
P. strobus	14	May 31	12.8	40.0	1.6	5.1	46.7	194.4	5.6	200.0	259.5
P. elliottii	5	June 21	4.7	9.0	14.0	1.5	24.5	25.6	0.5	26.1	55.3
P. taeda	4 & 8 (grafts)	June 22	13.0	12.6	18.4	5.4	36.4	47.0	1.8	48.8	98.2
Cr. japonica	4	June 3	6.9	2.6	0.4	2.6	5.6	267.3	5.7	273.0	285.5
M.glyptostroboides	10	June 6	3.7	23.4	0.8	4.7	28.9	42.8	3.5	46.3	78.9
Cu. lanceolata	8	June 28	3.5	1.8	0.1	0.7	2.6	204.3	2.8	207.1	213.2
Ch. obtusa	20	June 25	4.8	1.8	0.2	1.0	3.0	156.3	0.5	156.8	164.6

Notes: Factor Ⅰ showed an active zone of Rf 0.4~0.5, Factor Ⅱ, an active zone of Rf 0.6~0.7, and Factor Ⅲ, an active zone of Rf 0.2~0.5, respectively.

It will be observed from Table 1 that there was a clear difference in the amount of auxins according to fractions or tree species. Auxin concentrations in the neutral fraction were 3.0~13.0 μg IAA equivalents per 100 g fresh weight of shoots, and in general they were lower than the other two fractions. However, there was no remarkable difference in the auxin concentration among tree species. The acid fraction contained auxins equivalent to 2.6~46.7 μg IAA per 100 g shoots. Excepting *P. elliottii* and *P. taeda*, the bulk of auxins in the acid fraction was found in Factor Ⅰ which is presumed to be IAA. The amount of Factor Ⅰ was greater in *P. strobus* and *M. glyptostroboides*, mediate in the other four pines, and less in *Cr. japonica*, *Cu. lanceolata* and *Ch. obtusa*. On the other hand, Factor Ⅱ had very higher concentrations in *P. taeda* and *P. elliottii* as

compared with other species. The concentration of auxin in the aqueous fraction was equivalent to 23.1～273.0 μg IAA per 100 g shoots. The greater part of the aqueous auxins was Factor Ⅲ. The amount of Factor Ⅲ was more in *P. thunbergii*, *P. strobus*, *Cr. japonica*, *Cu. lanceolata* and *Ch. obtusa*, comparing with *P. densiflora*, *P. elliottii*, *P. taeda* and *M. glyptostroboides*. It has been definitely shown by above experimental results that the shoots of conifers contain auxins equivalent to a total of 41.1～285.5 μg IAA per 100 g fresh weight and that among the auxins the contents of water-soluble auxins are greatest in every species tested. However, these values, especially on Factor Ⅱ, may be lower estimates than the amount of promoters actually present in the extracts, since inhibitor β which exists together in the samples had inhibitory effect on the growth of pine hypocotyl sections.

2) Gibberellin-like substances in the shoots of conifers

Figure 7 shows the results of gibberellin bioassay by the rice seedling method using a dwarf variety "Tamanishiki". Although impurities in the extracts caused variation in both the Rf value and the tailing of the active substances on chromatograms, three zones with gibberellin activity were detected in each tree species, except for *P. thunbergii*, *P. strobus* and *Ch. obtusa*. The first zone was located at Rf 0～0.3, the second at Rf 0.4～0.5 and the third at Rf 0.8～1.0. These Rf values were different from those of known gibberellins. The first zone was especially striking in the fraction Ⅰ of *P. thunbergii*, while the third zone was not found in *P. thunbergii* and *P. strobus*. The second zone was detected in every species except *Ch. obtusa*, but the activity was generally lower. In the fraction Ⅰ of methanol extracts obtained from *P. densiflora* on May 2, on the other hand, a marked promoting zone was found at Rf 0.4～0.9 with a maximum peak at about Rf 0.6. This corresponded nearly to the position of gibberellin A, though having a broad promoting zone. Gibberellin-like activity was scarcely detected in *Ch. obtusa* because of a large quantity of inhibitors existing together. Except for the *Ch. obtusa* and the March 10 *P. densiflora* extract, most of inhibitors were detected in the fraction Ⅱ at Rf 0.5～1.0 with a maximum peak at Rf 0.6～0.8. The growth inhibiting activity was more pronounced in *P. thunbergii*, *P. strobus*, *Cu. lanceolata* and *Ch. obtusa* as compared with other five species tested.

Figure 8 shows the response of rice seedlings, a variety "Nōrin No. 22", to gibberellin-like substances in the extracts of shoots of five conifers.

Most of gibberellin-like substances were detected in the fraction Ⅰ. In this fraction there were promoting zones at Rfs 0～0.1, 0.2～0.3 and 0.6～0.8. The first zone was quite evident in *P. densiflora* and *P. elliottii*, only slight in *P. taeda*, and not detectable in *Cr. japonica* and *M. glyptostroboides*. The response of rice seedlings to the eluate from the zone of Rf 0～0.1 in *P. densiflora* is illustrated in photo. 1. The zones of Rf 0.2～0.3 and Rf 0.6～0.8 were recognized in all species tested. However, the former zone was more conspicuous in *P. densiflora* and *M. glyptostroboides*. Although the latter zone lay near the expected position of gibberellin A, its activity was relatively weak.

The approximate concentrations of gibberellins present in the shoots of conifers, which

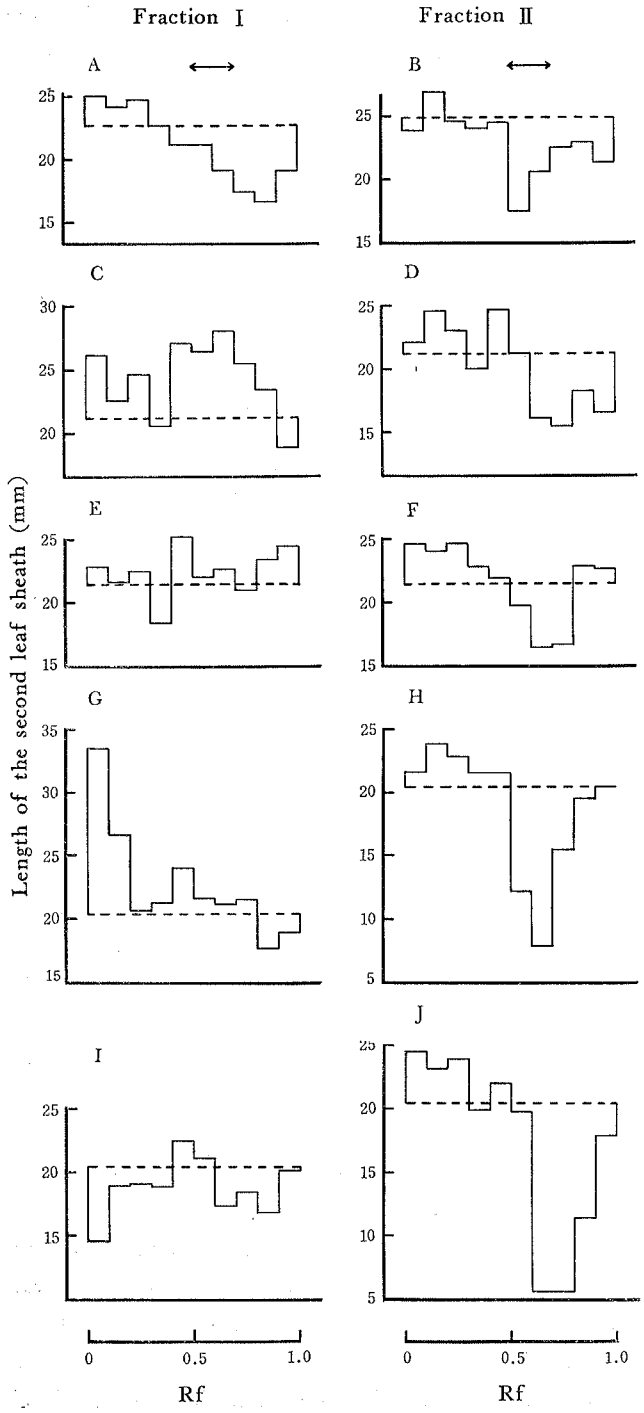

Fig. 7-A. Histograms showing gibberellin-like activity of extracts from the shoots of conifers. Chromatograms were developed with ammoniacal *iso*-propanol and assayed by the rice seedling test using a dwarf variety "Tamanishiki". Broken lines denote water controls. Arrows at the top of the histograms indicate the position of GA_3. Left column: Fraction I. Right column: Fraction II.

A~B, *P. densiflora* (March 10, 40 g); C~D, *P. densiflora* (May 2, 40 g); E~F, *P. densiflora* (June 29, 50 g); G~H, *P. thunbergii* (May 31, 50 g); I~J, *P. strobus* (May 31, 50 g).

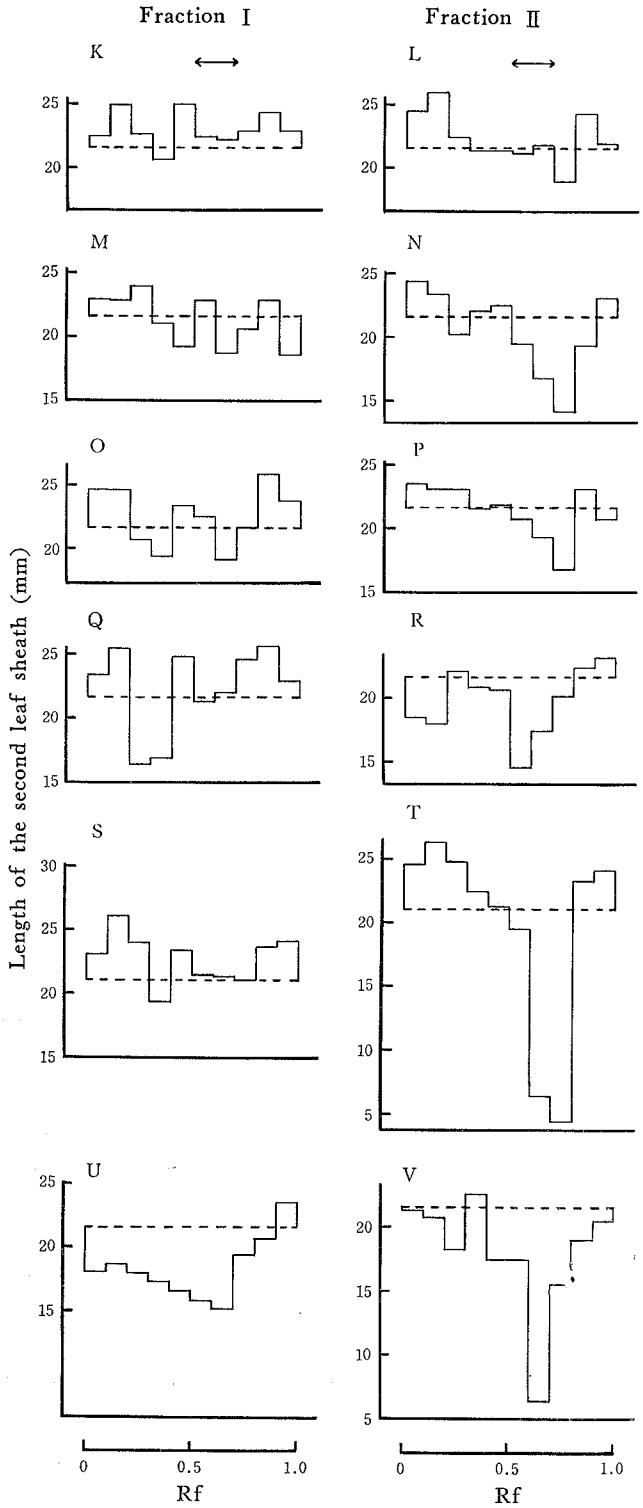

Fig. 7-B. K~L, *P. elliottii* (June 21, 50 g); M~N, *P. taeda* (June 22, 50 g); O~P, *Cr. japonica* (June 8, 50 g); Q~R, *M. glyptostroboides* (June 8, 50 g); S~T, *Cu. lanceolata* (June 28, 50 g); U~V, *Ch. obtusa* (June 30, 50 g).

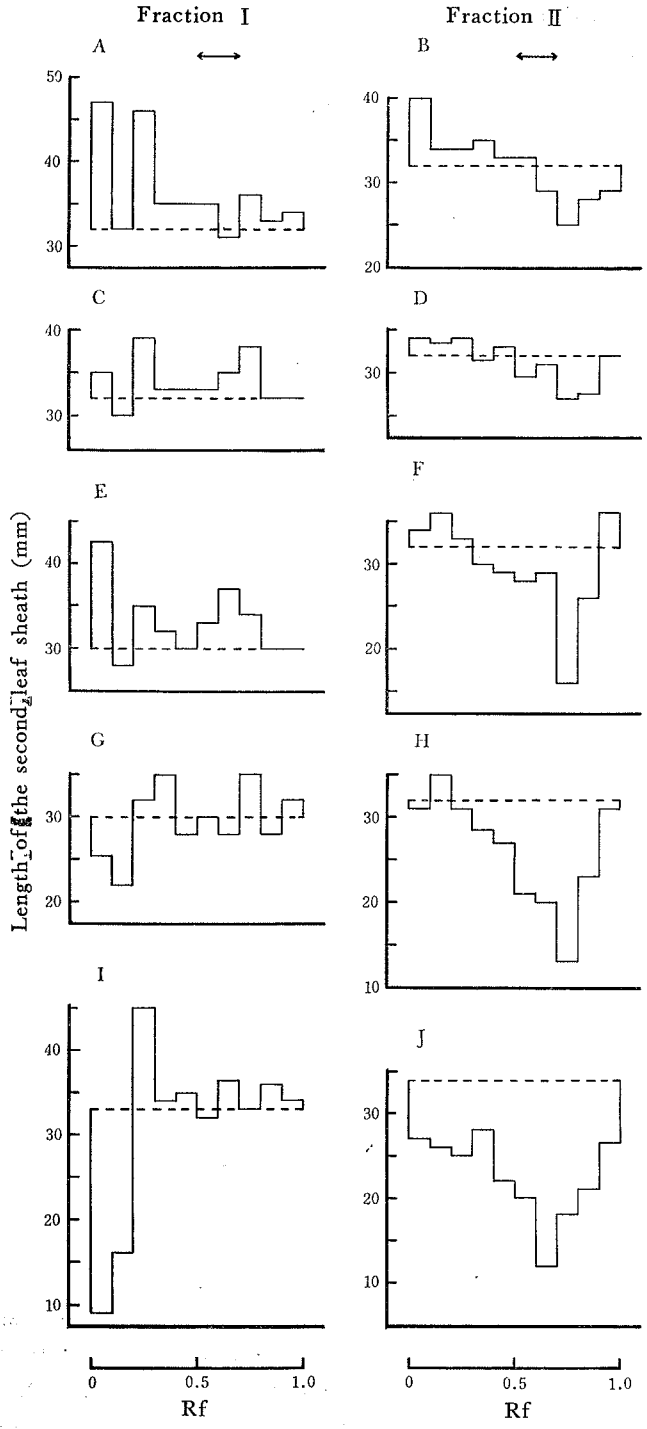

Fig. 8. Histograms showing gibberellin-like activity of extracts from the shoots of conifers. Chromatograms were assayed by the rice seedling test using a normal variety "Nōrin No. 22". Other descriptions are the same as those in Fig. 7-A.

A～B, *P. densiflora* (July 10, 50 g); C～D, *P. taeda* (July 10, 50 g); E～F, *P. elliottii* (July 10, 50 g); G～H, *Cr. japonica* (July 10, 50 g), I～J, *M. glyptostroboides* (July 24, 100 g).

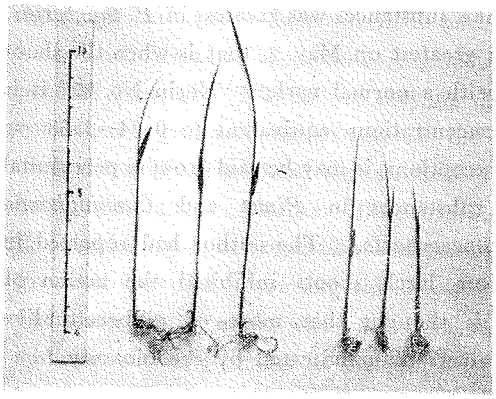

Photo. 1. Response of rice seedlings to a gibberellin-like substance obtained from *P. densiflora*.
Left, treated with the eluate from a chromatogram zone corresponding to Rf 0~0.1 in Fig. 8-A; right, control.

were calculated from the response curve of rice seedlings to gibberellin A_3 as shown in Fig. 3, are represented in Table 2.

Table 2. Gibberellin concentrations found in the shoots of conifers.

Tree species	Tree age	Date collected	Approximate concentration (μg. GA_3 equivalents/100 g.f.w.)			Rice varieties used for bioassay
			Fraction I	Fraction II	Total	
P. densiflora	5 & 8	March 10	0.04	0.01	0.05	
		May 2	0.35	0.13	0.48	
		June 29	0.14	0.16	0.30	
P. thunbergii	5	May 31	0.47	0.11	0.58	A dwarf mutant
P. strobus	14	May 31	0.02	0.17	0.19	"Tamanishiki"
P. elliottii	5	June 21	0.14	0.14	0.28	
P. taeda	4 & 8 (grafts)	June 22	0.07	0.07	0.14	
Cr. japonica	4	June 8	0.16	0.06	0.22	
M. glyptostroboides	10	June 8	0.20	0.02	0.22	
Cu. lanceolata	8	June 28	0.24	0.27	0.51	
Ch. obtusa	20	June 30	0.02	0.01	0.03	
P. densiflora	5 ~ 7	July 10	1.35	0.28	1.63	
P. taeda	3	〃 〃	0.36	0.11	0.47	
P. elliottii	4	〃 〃	0.85	0.17	1.02	"Nōrin No. 22"
Cr. japonica	20	〃 〃	0.28	0.04	0.32	
M. glyptostroboides	9	June 24	0.24	0	0.24	

From Table 2, it is recognized that there is some difference on the amount of gibberellin-like substances according to tree species, the time of extraction or the kind of test plants. When assayed with a dwarf variety "Tamanishiki", gibberellin-like substances equivalent to 0.03~0.58 μg gibberellin A_3 per 100 g fresh weight were detected in the

shoots of conifers. The amount of gibberellin-like substances was greatest in *P. thunbergii*, and least in *Ch. obtusa*. In *P. densiflora* it was greatest on May 2, that is when the shoot growth was most active. On the other hand, with a normal variety "Nōrin No. 22" it is shown that gibberellin-like substances had concentrations equivalent to 0.24~1.63 μg gibberellin A_3 per 100 g shoots. With some exceptions, it may be said from experimental results that the contents of gibberellin-like substances in *Pinus* and *Cunninghamia* are greater than those in *Cryptomeria* and *Chamaecyparis*. The author had reported in previous paper that inhibitor β obtained from larch shoots inhibited the action of gibberellin on rice seedlings.[10] Therefore, it is thought that more of gibberellin-like substances may be present in the shoots of conifers than indicated by the bioassay.

3. Discussion

Chromatographic surveys of auxins in the buds or shoots of conifers have already been carried out by Fransson,[11] Allen,[12] Ueda et al.,[13] Ogasawara,[14~17] Hashizume,[18] Yim,[19] Saito et al.,[20] Clark et al,[21] etc. In these studies, many investigators have reported that IAA exists in many conifers as a native auxin. Ogasawara[14~17] studied chromatographically growth substances in buds of *Pinus* species. As a result, several auxins were detected on the chromatogram of the acid fraction of ether extracts. One of them corresponded to the Rf value of IAA but the others had Rf values smaller than IAA, when developed with ammoniacal *iso*-propanol. He also found that the activity of an auxin corresponding to the position of IAA was considerably increased by treating pine buds with tryptophane which is a precursor of IAA. However, Fransson[11] reported that a conspicuous promoter in the acid ether fraction of seedling shoot extract of *P. silvestris*, termed "*Pinus* 1", has a Rf value somewhat smaller than that of IAA and its physiological property differs evidently from that of IAA. On the other hand, Saito and Shibakusa[20] reported that most of the auxins in the buds of *Abies sachalinensis* during sprouting season were found in the aqueous fraction of ethanol extracts. Furthermore, Hashizume[22] reported that a remarkable growth prompting substance present in pine seeds is a water soluble auxin.

In the present experiment, the following three kinds of remarkable growth promoting activities were detected in methanol extract from shoots of conifers. It seems probable that these growth promotions are due to auxins and not to gibberellins, because the gibberellins are inactive on the pine hypocotyl test. Factor Ⅰ, detected at Rf 0.4~0.5 on the chromatogram of the acid fraction, is probably IAA as compared with the Rf value of synthetic IAA in the guide chromatogram, although it gave color reaction neither with Ehrlich's nor Sakowski's reagents. Factor Ⅱ, found in the acid fraction at Rf 0.6~0.7, has a very similar Rf value to "Promoter 3" which has been detected by Allen[12] in the buds of *P. palustris*, but its chemical nature is not confirmable. Factor Ⅲ, detected at Rf 0.2~0.5 on the chromatogram of the aqueous fraction, nearly corresponds in Rf value to a water-soluble auxin which has been found in the buds of *Abies sachalinensis* and pine seeds. However, it is considered that the factor may consist of some growth substances for giving relatively wide distribution on the chromatogram.

Although the water-soluble auxin in pine seeds produced characteristic indole colors with both Ehrlich's and Salkowski's reagents, that in coniferous shoots failed to give the positive reaction. The failure may be due to the presence of color-inhibiting substances contained together in the extacts of the shoots. The Factor Ⅲ was negative to both ninhydin and ammoniacal silver nitrate reagents, and it was soluble in water and alcohols but not in ether. Further, the author observed in another experiment that the Factor Ⅲ inhibits the root formation and growth of *Oryza sativa*. From these results, it seems probable that the promoting substance is not amino acids or sugars but an auxin similar to a water-soluble auxin which was detected in pine seeds. The occurrence of ether-insoluble and water-soluble auxins in higher plants, some of which are IAA-sugar conjugates, have been reported by Audus,[23] Gunning,[24] and Srivastava.[6] The present experiment failed in the identification of the water-soluble auxin in conifers. However, since the water-soluble auxin is found in abundance in coniferous shoots, it is thought that it plays an important role in the growth and develoment of conifers as a native auxin in the same way as ether-soluble auxins in the acid fraction.

In the acid fraction of extracts from conifers, usually some promoting substances have been detected at Rf values smaller than IAA when assayed by the *Avena* straight growth test. However, they were hardly observed in the present experiment, assayed in the pine hypocotyl test. Although it is hard to understand the reason, it is thought that the substances may be inactive in pine hypocotyl sections or that it is removed when the ether layer is washed with water, because they may be false auxins. Srivastava[6] has found that, if the ether fractions were washed with water and dried over anhydrous Na_2SO_4 prior to paper chromatography the contaminants like sugars or amino acids, which sometimes promote elongation of *Avena* coleoptile sections, were not detected on the chromatograms.

Although the presence of gibberellin-like substances in higher plants has been reported by many investgators, studies on gibberellin-like substances in gymnosperms are very few in number. The first evidence for the occurrence of gibberellin-like substances in conifers was obtained by Kato et al.[25] They reported that extracts of berries of *Juniperus chinensis* proved active in promoting the growth of the dwarf-3 and dwarf-5 mutants of *Zea mays*. After that Saito and Shibakusa[20] found the presence of gibberellin-like substances in extracts from buds of *Abies sachalinensis*, applying the paper chromatography. Moreover, Hashizume[10] ascertained from the paper chromatography and the rice seedling test that several kinds of gibberellin-like substances are contained in methanol extract from new shoots of *Larix leptolepis*. Very recently, Kurgman[26] reported that a gibberellin-like substance was isolated and identified from the immature seeds of three pine species.

In the present experiment, at least four kinds of gibberellin-like activities were detected at Rfs 0～0.3, 0.4～0.5, 0.6～0.8 and 0.8～1.0 on the chromatogram of methanol extract from coniferous shoots. Among these, the active zone of Rf 0.6～0.8 corresponds to Ogawa's *Pharbitis* factor Ⅰ,[27] which is extremely abundant in immature seeds of convolvulus

plants. It has been found also in the extracts from buds of *Abies sachalinensis*,[20] shoots of *Larix leptolepis*[10] and immature seeds of *Robinia pseudo-acacia*.[28] Probably its activity is attributed to the known gibberellin A. The active zone of Rf 0.4～0.5 nearly coincides in Rf value with Ogawa's *Pharbitis* factor II,[27] and a gibberellin-like substance of *Prunus persica*,[29] and Murakami's gibberellin A_3 glucoside synthesized in living plant tissues.[30] It has been detected also in extracts from new shoots of *Larix leptolepis*. And the present experiment demonstrated that the acivity of this active zone rose when a large quantity of gibberellin A_3 was supplied in excised shoots of *Cryptomeria japonica*. Therefore, it is considered that the substance existing in an active zone of Rf 0.4～0.5 may be one of gibberellin A glycosides. The active zone of Rf 0～0.3 has a very similar Rf value to that of Ogawa's *Pharbitis* factor III.[27] In woody plants, it has been found in buds of *Abies sachalinensis*, new shoots of *Larix leptolepis*, and immature seeds of *Wistaria floribunda* and *Maackia amurensis*, especially in the latter two in large amounts.[28] Murakami[31] reported the presence of water-soluble gibberellins in the aqueous fraction of extracts of seeds of *Pharbitis nil* and *Wistaria floribunda*. The substances are detected at Rf around 0.2 and Rf around 0.4, when developed with ammoniacal *iso*-propanol. According to Ogawa[32] and Hashizume,[10] however, the gibberellin-like substances of lower Rf values in seed of *Lupinus luteus* or new shoots of *Larix leptolepis* are not always the water-soluble gibberellin and they are detected also in the acid fraction. The active zone of Rf 0.8～1.0 has also been detected in extracts from new shoots of *Larix leptolepis*. However, its chemical nature is not known. Recently, Tamura et al.[33] reported that the substance without the gibbane ring such as helminthosporol or kaurenoide is active in gibberellin bioassay. Therefore, there is a possibility that such compounds may be contained in gibberellin-like substances detected in the present experiment.

Some examples of auxin concentrations found in the organ or tissue of various flowering plants are described in Leopold's book.[34] According to his book, the greatest quantity of auxin is found in the endosperm of the corn, which is equivalent to 10,500 μg IAA per 100 g fresh weight. Further the amount of auxin per 100 g fresh weight is estimated to be 7.4 μg IAA equivalents in stems of the sunflower and 1.1 μg IAA equivalents in young leaves of the pineapple, respectively. The total amounts of auxins present in coniferous shoots were equivalent to 41～286 μg IAA per 100 g fresh weight as shown in the present experiment. Accordingly, it seems that the coniferous shoots are comparatively abundant in the content of auxin.

Radley, McComb and Carr, Corcoran, Hirono et al., Murakami, and Ogawa have estimated amounts of extractable gibberellin-like substances obtained from various parts of flowering plants.[35] Murakami[36] reported that the seeds of the *Convolvulaceae* contained considerably higher concentrations of gibberellins among 18 species examined and their contents were equivalent to 10～100 μg gibberellin A per 100 g of dry seed. Diffusates from the seeds of *Malus pumila*, however, had an equivalent activity of 0.3 μg gibberellin A per 100 g dry seed. Hirono et al.[37] estimated ethyl alcohol diffusates from the young

fruit of *Prunus persica* to contain 150 μg gibberellin A_3 equivalents per 100 g fresh weight. Very recently, Kurgman[26] reported that the maximum concentration of gibberellin-like substance in immature sugar pine seeds is equivalent to 0.4 μg gibberellin A_3 per 100 g dry weight. In the present experiment, the amounts of gibberellin-like substances in growing shoots of conifers were calculated to be equivalent to 0.03~1.63 μg gibberellin A_3 per 100 g fresh weight. Therefore, it may be said that conifers contain relatively lower concentrations of gibberellins as compared with other flowering plants.

Ⅲ. Relation between flower bud formation and endogenous growth substances in conifers

It has been known that many of the conifers growing under natural conditions usually attain reproductive maturity after the lapse of a certain year and start flower formation, though the flowering habit differs somewhat with tree species. Kato et al.,[38] Shidei et al.,[39] Hashizume[1] etc., however, found that seedlings or young trees of several species belonging to *Taxodiaceae* and *Cupressaceae* were flowered by spraying with gibberellin. Recently, the author also observed that flowering in comparatively young trees of *Cryptomeria japonica* and *Larix leptolepis* was remarkably promoted by girdling or binding. In the former case in which flower formation occurred naturally, it is considered that the tree age is closely related with flower formation in conifers and physiological condition for flower formation is gradually made in the tree body with the passing of age. In the latter case in which flower formation was artificially induced, it is presumed that artificial treatments cause a sudden change of physiological condition in the body of the tree and as the result the transition from vegetative to reproductive growth occurs. In every case mentioned above, however, the physiological mechanism of flower formation is not made clear enough. Since flower initiation in conifers occurs by spraying with plant hormones like gibberellins, it is imagined that growth substances present in the tree body will play an important role to flower formation. This experiment was designed to make clear the relation between flower formation and endogenous growth substances.

1. Materials and methods

In order to induce flower bud formation, the following treatments were practiced.

Girdling: The treatment was done on July 13 on side branches of 8-year-old *Cryptomeria japonica* (grown from cuttings). Bark and cambium were removed in a circle of 2 cm width around the branch at the basal part of side branches. The removed bark was turned upside down, again set in the girdled part, and then bound with waxen paper tape.

Foliar spray of gibberellin: 3 and 4 years old *Cr. japonica* used as the materials. In the first experiment using 4 year-old trees, a water solution containing 200 ppm gibberellin was sprayed 3 times during August 10 to 12 over shoots on side branches. In the second experiment using 3 year-old trees, the spray of gibberellin at the concentration of 300 ppm

was done during September 22 to 24 3 times. Both girdling and gibberellin spray were treated on one side branches of sample trees, and another side untreated branches which are no flowering were used as the controls. After the treatments, new shoots of the portion bearing flowers were collected according to the process of flower bud formation, and were used for extraction.

To study the metabolism of exogenous gibberellin in coniferous tissues, growing shoots of *Cr. japonica* (15 cm in length) were cut off from an about 20 year-old tree on September 6. The excised shoots were planted in a beaker holding a water solution of 500 ppm gibberellin A_3 and incubated under natural temperature and light conditions. After 2, 6, and 10 days, 50 g of the shoots were taken out from the solution, washed with water, and used for extraction.

To investigate the relationship between tree age and endogenous growth substances, moreover, on Septmber 26 samples for extraction were collected from the current year-old seedlings, and 1, 5 and 13 years old trees of *P. densiflora*, respectively.

In these experiments, 20 g of samples (in fresh weight) were used for auxin extraction and the chromatogram strip corresponding to the extract from 5 g samples was assayed by the pine hypocotyl test. On the other hand, for extraction of gibberellin-like substances 40 g (an experiment shown in Fig. 11) or 50 g (experiments shown in Figs. 14 and 15) of samples were prepared. Methods for extraction, separation and bioassay of auxin and gibberellin-like substances were the same as those described in the first experiment (see Ⅱ－1), except for an experiment. In an experiment shown in Fig. 14, to separate gibberellin-like substances and inhibitors the extracts of new shoots were first developed by the ascending method with *n*-butanol, ammonium hydroxide, and water (100 : 3 : 18, v/v/v). The developed chromatograms were divided into two parts corresponding to Rf 0～0.4 (fraction Ⅰ) and Rf 0.4～1.0 (fraction Ⅱ), and each part was eluted separately with methanol. Each eluate was concentrated to come a small amount, and then spotted on chromatographic papers (Tōyō No. 50) and again developed with *iso*-propanol, ammonium hydroxide, and water (8 : 1 : 1,v/v/v).

2. Results

1) Changes of growth substances in new shoots of *Cr. japonica* in relation to flower induction by girdling

After girdling the shoots showed a tendency of wilting and the growth became slowly. In about 30 days after the treatment, i. e. the middle of August, a sign of flower bud formation was seen in a large majority of the treated shoots, and during early in September the setting of flower buds was externally observed.

Changes in endogenous growth substances occurring in new shoots in relation to flower induction by girdling are shown in Figs. 9～11 and Table 3.

From Fig. 9, on the chromatograms of the acid fraction two considerable zones showing auxin activity were seen at Rf 0.4～0.5 (Factor Ⅰ) and Rf 0.6～0.7 (Factor Ⅱ), and

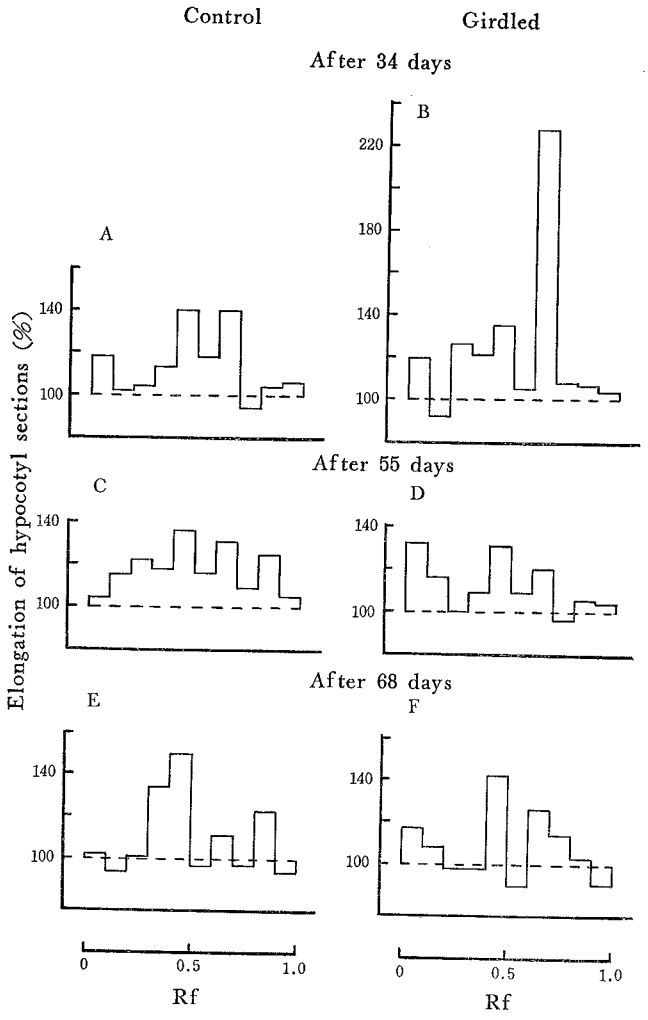

Fig. 9. Histograms showing changes in auxin of the acid fraction occurring in new shoots of *Cr. japonica* in relation to flower induction by girdling.

Left column (A, C, and E), untreated controls; right column (B, D, and F), girdled shoots. A~B, August 16 (34 days after girdling); C~D, September 6 (55 days after girdling); E~F, September 19 (68 days after girdling).

two weak promoting zones, at Rf 0~0.1 and Rf 0.8~0.9. The Factor I which is probably IAA tended gradually to decrease after girdling as compared with untreated controls, while the Factor II increased suddenly and abnormally on the 34th day after girdling, i. e. at the early stage of flower bud formation. On and after the 55th day, however, it decreased rapidly and differed little from the untreated control. A promoting zone of Rf 0~0.1 was seen to have disappeared on the 55th and 68th days in controls, while it remained in girdled shoots on the same days of inspection. A promoting zone of Rf 0.8~0.9 was hardly found in treated shoots. Consequently, as seen in Table 3, the amount of total auxin in the acid fraction was greatest in the girdled shoots after 34 days and corresponded to 163.6 μg of IAA per 100 g fresh shoots. The amount of acid auxin in the girdled shoots fell rapidly after that, and on and after the 55th day it was less than that in untreated controls.

In the aqueous fraction (Fig. 10), on every chromatogram of the control and the treatment a promoting zone was detected in a wide range of Rf 0~0.6 with a maximum

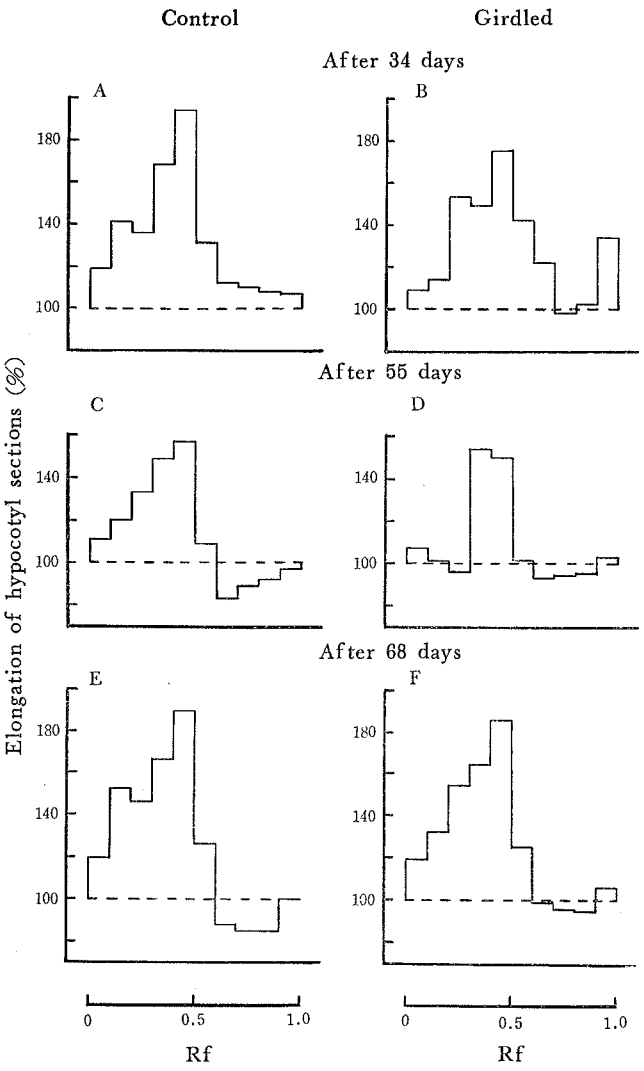

Fig. 10, Histograms showing changes in auxin of the aqueous fraction occurring in new shoots of *Cr. japonica* in relation to flower induction by girdling. Other descriptons are the same as those in Fig. 9.

peak at Rf 0.4~0.5. Although the nature of aqueous auxins was hardly changed by girdling, their amount in girdled shoots tended to decrease after the treatment as compared with that of untreated controls (see Table 3).

As shown in Fig. 11, several gibberellin-like substances were contained in the extracts from new shoots of *Cr. japonica*. The bulk of gibberellin-like activities was found in the fraction Ⅰ, while inhibiting activities were detected in the fraction Ⅱ and most of them were found out to be inhibitor β termed by Bennet-Clark and Kefford.[41] On chromatograms from untreated shoots, four zones with gibberellin-like activity were detected at Rfs 0~0.3, 0.4~0.5, 0.6~0.8, and 0.9~1.0. In girdled shoots, however, the activity of these promting zones, except for a zone of Rf 0.9~1.0, tended to decrease on and after the 34th day after the treatment, and two promoting zones of Rf 0~0.3 and Rf 0.6~0.8 disappeared after 55 days. On the other hand, the inhibitor β tended

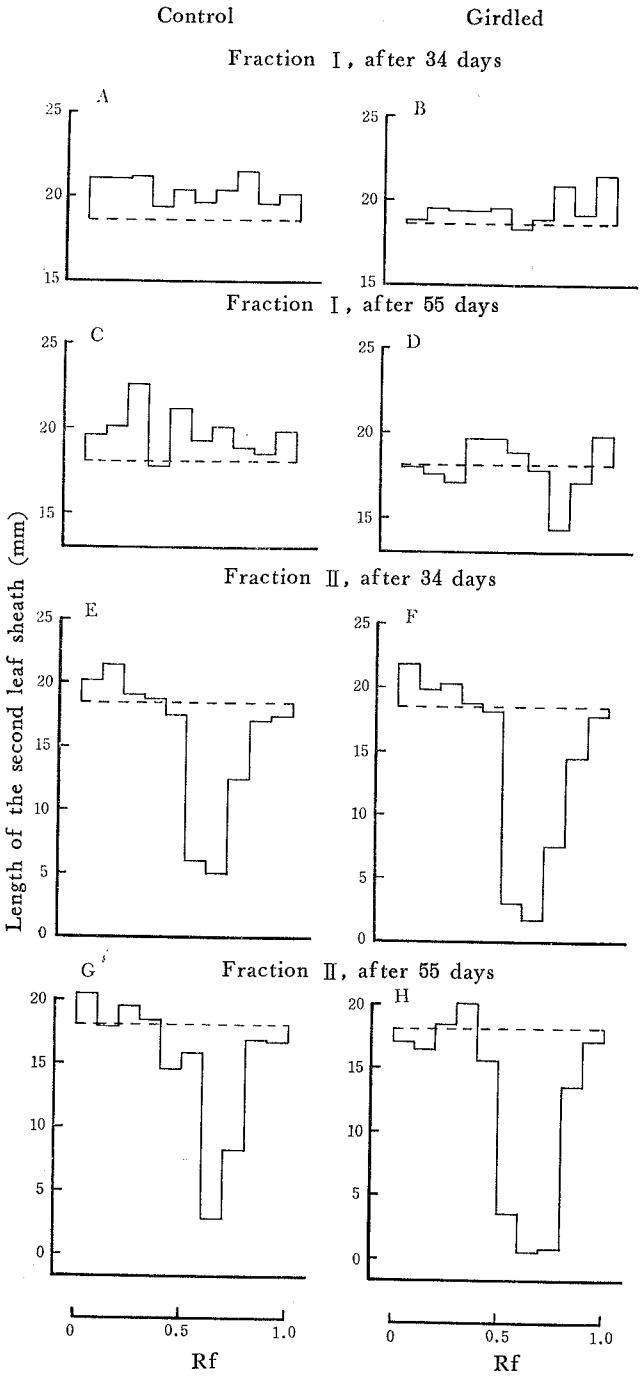

Fig. 11. Histograms showing changes in gibberellin-like substances and inhibitors occurring in new shoots of *Cr. japonica* in relation to flower induction by girdling. The extract from 40 g shoots was assayed by the rice seedling test using a dwarf variety "Tamanishiki".

Left column, untreated controls; right column, girdled shoots. A~D, fraction I; E~H, fraction II. A~B and E~F, August 16 (34 days after girdling); C~D and G~H, Septembr 6 (55 days after girdling).

to increase markedly in girdled shoots. This tendency was plainly recognized on the chromatograms of the 55th day after girdling. From these results, it is concluded that the girdling treatment causes the fall of the level of gibberellin-like substances and the rise of inhibitor level in new shoots.

Table 3. Changes in amount of auxin in new shoots of *Cr. japonica* caused by girdling.*

Date of collection	Approximate concentration (μg. IAA equivalents/100 g.f.w.)				Remarks
	Acid auxin		Agueous auxin		
	Control	Girdled	Control	Girdled	
After 34 days	4.5	163.6	69.9	30.2	An indication of flower formation was recognized
After 55 days	4.9	3.4	14.3	11.2	Flower buds were plainly visible
After 68 days	6.9	3.6	61.4	46.9	

*The girdling was done on July 13.

2) Changes of growth substances in new shoots of *Cr. japonica* in relation to flower induction by spraying with gibberellin

When gibberellin was sprayed in August, in about 25 days after the treatment the origin of flower buds was recognized in the tip of the treated shoot, and after 40 days flower buds were plainly visible.

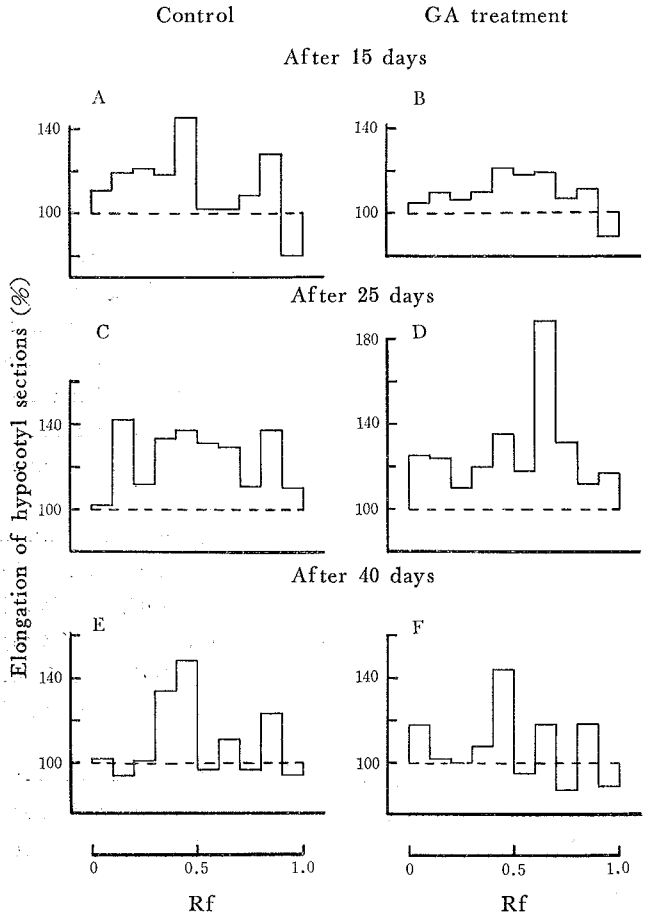

Fig. 12. Histograms showing changes in auxin of the acid fraction occurring in new shoots of *Cr. japonica* in relation to flower induction by spraying with gibberellin.

Left column (A,C, and E), untrated controls; right column (B, D, and F), shoots sprayed with gibberellin. A~B, August 25 (15 days after spraying); C~D, September 4 (25 days after spraying); E~F, September 19 (40 days after spraying).

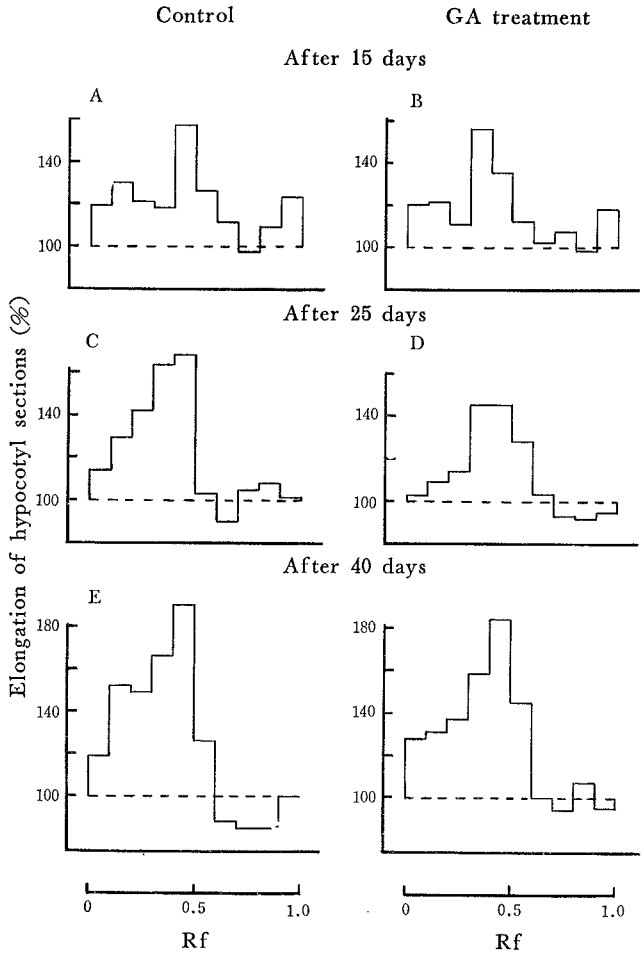

Fig. 13. Histograms showing changes in auxin of the aqueous fraction occurring in new shoots of *Cr. japonica* in relation to flower induction by spraying with gibberellin. Other descriptions are the same as those in Fig. 12.

Table 4. Changes in amount of auxin in new shoots of *Cr. japonica* caused by spraying with gibberellin.*

Date of collection	Approximate concentration (μg. IAA equivalents/100 g.f.w.)				Remarks
	Acid auxin		Aqueous auxin		
	Control	Treated	Control	Treated	
After 15 days	4.7	2.0	12.6	11.0	
After 25 days	9.2	39.2	27.1	5.3	Initiation of flower buds was recognized
After 40 days	6.0	3.3	62.9	34.9	Flower buds were plainly visible

*The foliar spray of gibberellin at the concentration of 200 ppm was done 3 times during the period of August 10~12.

Changes in auxins caused by spraying with gibberellin are shown in Figs. 12~13 and Table 4. As for the acid fraction, in controls considerable promoting zones were found at Rf 0.4~0.5 (Factor I) and Rf 0.8~0.9 on every chromatogram and moreover at

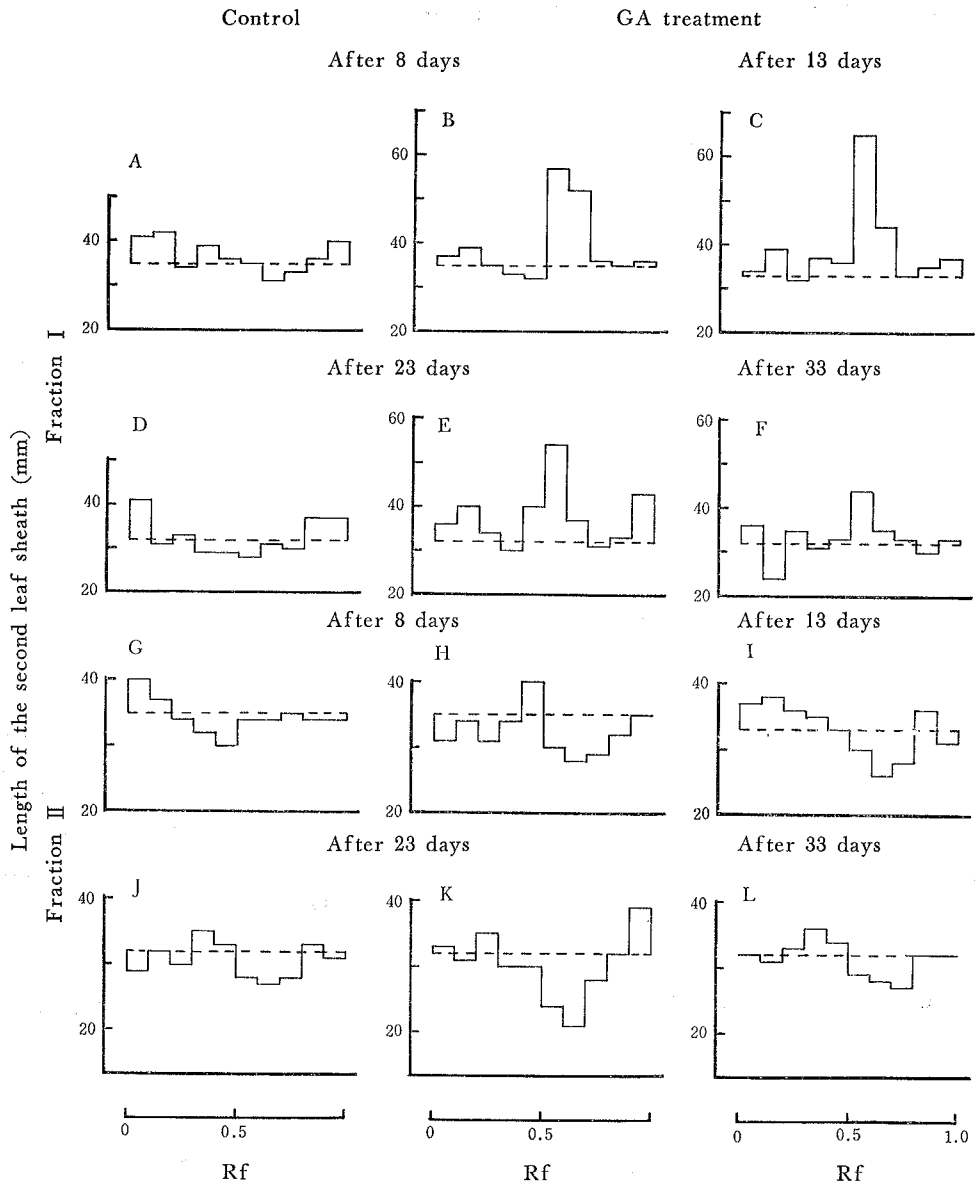

Fig. 14. Histograms showing changes of gibberellin-like substances and inhibitors occurring in new shoots of *Cr. japonica* in relation to flower induction after being sprayed with gibberellin at a concentration of 300 ppm. Methanol extracts of 50 g shoots were first developed with ammoniacal *n*-butanol, eluted, and rechromatographed with ammoniacal *iso*-propanol. The bioassay was performed with the rice seedling test using a variety "Nōrin No. 22".

A, D, G, and J, untreated controls; the others, shoots sprayed with gibberellin. A~F, fraction I; G~L, fraction II. A~B and G~H, September 30 (8 days after spraying); C and I, October 5 (13 days after spraying); D~E and J~K, October 15 (23 days after spraying); F and L, October 25 (33 days after spraying).

Rf 0.1～0.2 on the chromatogram of the 25th day. In the treatment, Factor I and a promoting zone of Rf 0.8～0.9 tended to fall in the activity at the time of flower induction, i. e. on the 15th and 25th days after spraying. After 40 days still they remained at lower levels of activity, comparing with the control. However, the most surprising change in auxin in the shoot sprayed with gibberellin is that a remarkable promoting zone at Rf 0.6～0.7 appeared abruptly on the 25th day after spraying. The zone vanished mostly on the 40th day after spraying. Consequently, as seen in Table 4, the amount of acid auxin in treated shoots was less than that in untreated controls on the 15th and 40th days after spraying, but on the 25th day it increased to about four times of the control.

In the aqueous fraction (Fig. 13), auxin activity was detected at Rf 0～0.6 on every chromatogram of the control and the gibberellin treatment, being a peak at Rf 0.3～0.5. As seen from the histograms, there was no distinct difference in kind of auxin between the control and the treatment. However, the amount of auxin was less in the treatment than in the control as shown in Table 4. Auxin content in treated shoots fell conspicuously on the 25th day after spraying; at the early stage of flower bud formation.

Changes in gibberellin-like substances and inhibitors in new shoots by treatment with gibberellin are shown in Fig. 14. On the chromatograms of untreated controls, weak gibberellin-like activities could be deteced only near the starting line and the solvent front, Probably this is due to reasons that the material used for extraction and the time of its collection in the present experiment differed from those in the before-mentioned experiments. On the chromatogrms from treated shoots, however, a remarkable promoting zone was found at Rf 0.5～0.7 in the fraction I. This promotion is perhaps due to gibberellin A absorbed through the surface of new shoots. Although the substance fell gradually with the time elapsed, it remained in a considerable degree in treated shoots even on the 33th day after spraying. In treated shoots, substances with pronounced gibberellin activity, except for gibberellin A absorbed, were not recognized and the formation of native gibberellin-like substances also was unaffected by exogenous gibberellin. From these results, it may be infered that gibberellin A absorbed into new shoots is not converted into other gibberellin-like substances, but is directly consumed in that condition. As shown by the histograms of the fraction II, inhibitor β tended to increase after spraying with gibberellin as compared with untreated controls.

3) Metabolism of absorbed gibberellin A_3 in excised shoots of *Cr. japonica*

The author described above that gibberellin A absorbed through the foliar surface shall be consumed in that condition in new shoots of *Cryptomeria*. Murakami,[30] however, observed that exogenous gibberellin A_3 is converted in many plants into a glycoside which induces a growth response in the rice seedling test. So the present experiment was made to solve such a divergence of experimental results ; a solution of gibberellin A_3 at higher concentration was continuously absorbed through the cutting section of excised shoots of *Cr. japonica* and the metabolism of gibberellin A_3 in the shoots was

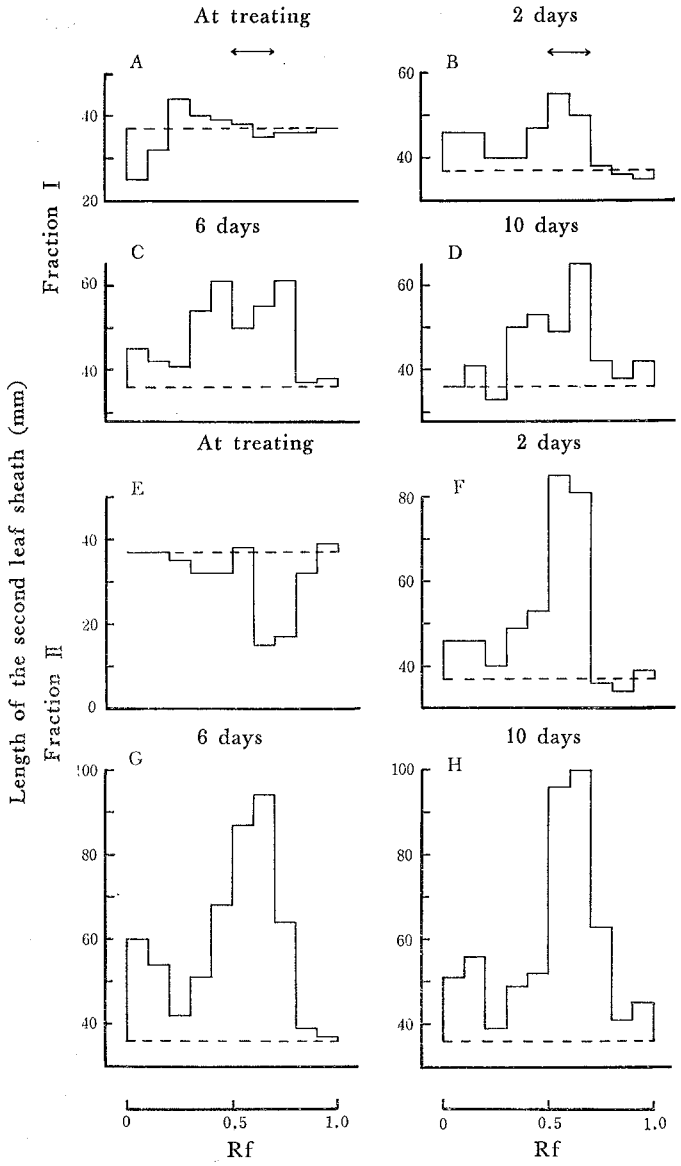

Fig. 15. Histograms showing changes of gibberellin-like substances in excised shoots of *Cr. japonica* treated with GA_3 at a concentration of 500 ppm. Bioassay was performed by the rice seedling test using a variety "Nōrin No. 22". Days on the histograms represent the time after treatment with gibberellin. Arrows at the top of the histograms indicate the position of GA_3. A~D, fraction I; E~H, fraction II.

investigated by the paper chromatography (Fig. 15). Though distiributing in a relatively wide zone on chromatogram, absorbed gibberellin A_3 was mainly detected in the fraction II at Rf 0.5~0.7, and only a little in the fraction I. In the fraction I, however, another noticeable active zone was observed at Rf 0.3~0.5 on the chromatograms of the 6th and 10th days after the treatment. The substance showing the promotion is likely to be identical with gibberellin A_3 glucoside, judging from Murakami's investigation. Further, a weak gibberellin-like substance was detected at Rf 0~0.2 on the chomatograms from the treated shoots. It was more evident on the chromatograms of the 6th and 10th days after the treatment, though its chemical nature could not be established.

Comparing with the experiment of the foregoing paragraph, it is considered that

superfluous gibberellin A_3 may be converted in the shoots of *Cryptomeria* into gibberellin derivatives such as gibberellin A_3 glucoside when applied in large quantities.

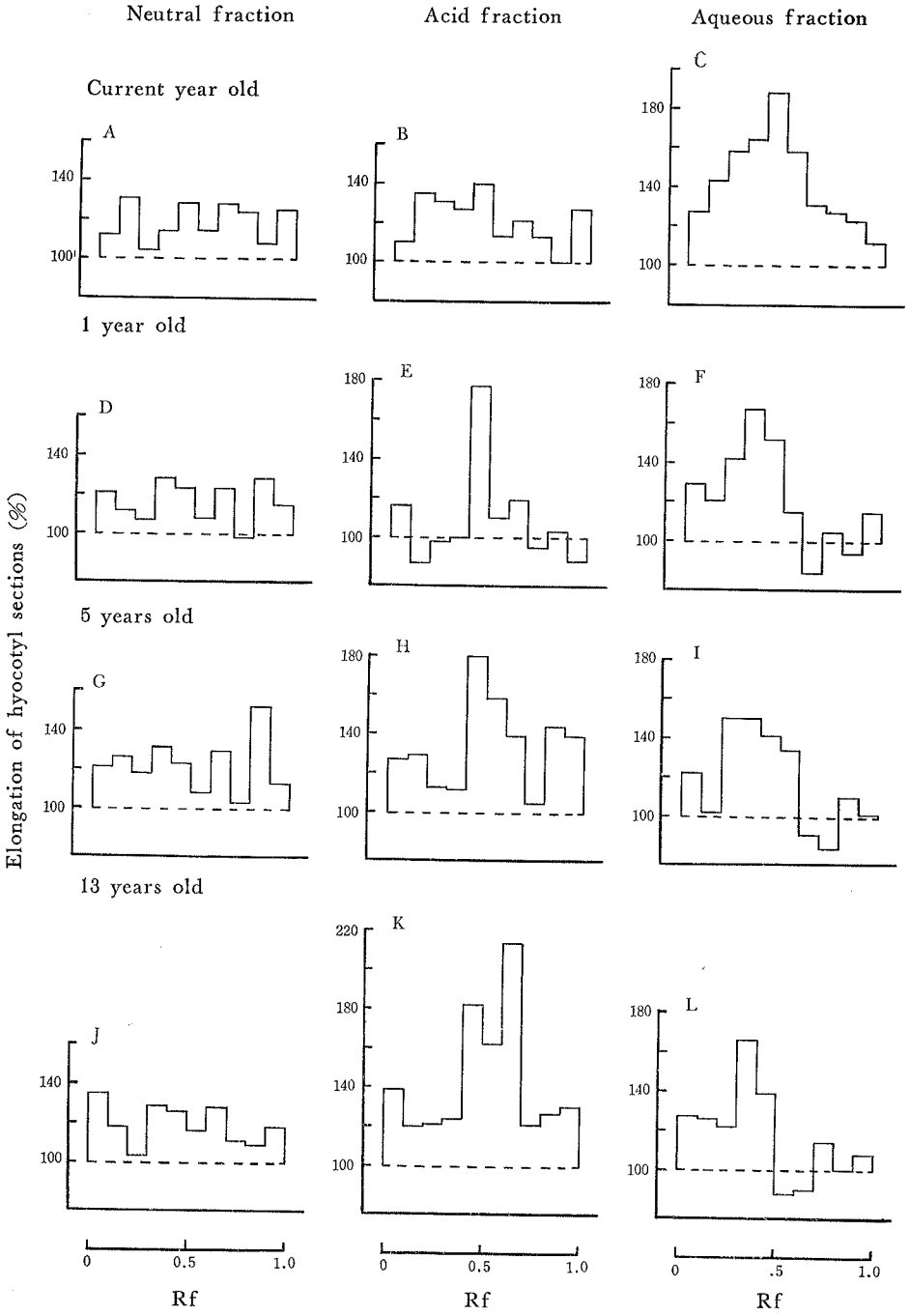

Fig. 16. Changes of auxins in the buds of *P. densiflora* in relation to tree age. Left column, neutral fraction; central column, acid fraction; right column aqueous fraction. A~C, current year old; D~F, 1 year old; G~I, 5 years old; J~L, 13 years old.

Table 5. Relation between the age of tree and the amount of auxin in the buds of *P. densiflora*.*

Age of tree (year)	Approximate concentration (μg. IAA equivalents/100 g.f.w.)							Remarks
	Neutral auxin	Acid auxin				Aqueous auxin	Total auxin	
		Factor I	Factor II	Others	Total			
0.5**	5.5	1.7	0.6	5.0	7.3	59.1	71.9	Stage of primary leaves; needles were not yet formed
1	4.8	17.0	0.4	0.3	17.7	22.1	44.6	
5	10.8	18.4	1.7	14.5	31.6	13.9	59.3	
13***	5.8	19.2	111.4	11.9	142.5	18.9	167.2	Flower bearing was abundant

* The materials used for extraction were collected on September 26.

** Young shoots were used for extraction.

*** Trees propagated by grafting.

4) Relation between tree age and auxin in *P. densiflora*

The relation between tree age and endogenous auxin is as shown in Fig. 16 and Table 5. In the neutral fraction there were promoting zones at Rfs 0~0.2, 0.3~0.5, 0.6~0.7, and 0.8~1.0. The activity of these promoting zones, however, did not very vary with tree age. Therefore, there was no appreciable difference in the content of neutral auxin among tree ages (see Table 5). Although the acid fraction contained several promoting zones, two zones of Rf 0.4~0.5 (Factor I) and Rf 0.6~0.7 (Factor II) were especially remarkable. Factor I was found in very little concentration corresponding to 1.7 μg IAA per 100 g shoots in the current year old seedlings, but its concentration increased at a bound to ten times in one year old trees and tended to increase slowly with the increase of tree age. Factor II existed in relatively lower concentrations in trees below five years old, but in 13 years old trees it increaed extremely and showed a concentration equivalent to 111.4 μg IAA per 100 g shoots. Consequently, as shown in Table 5, the total amounts of acid auxins increased rapidly with the increase of tree age. In the aqueous fraction, auxin activities could be detected in a wide range of Rf 0~0.6. The concentration of aqueous auxin, as shown in Table 5, was the highest in the current year's seedlings, but in one year old trees it fell at a stroke to about one-third of them. Among 1~13 years old trees, there was no remarkable variation in the content of aqueous auxin.

3. Discussion

As has been already reported, applied gibberellin induces the flower formation of many species of *Taxodiaceae* and *Cupressaceae*, but it is not effective to the flower induction of *Pinaceae* species. Comparing growth substances contained in *Pinaceae* species with those of *Taxodiaceae* and *Cupressaceae* species for elucidating physiologically the reason, no regular relation was found in the total amounts of auxins between above two groups. Two promoting zones, the Rf 0.5~0.8 in the neutral fraction, and the Rf 0.6 ~0.7 in the acid fraction, however, were especially evident in *Pinus* species. As to

gibberellin-like substances and growth-inhibiting substances, on the other hand, there were found no qualitative and quantitative differences between the above two tree groups, though it has been known from another experiment of the author that inhibitor β obtained from larch shoots checks the action of gibberellin on rice seedlings. With only these facts, it is difficult to explain completely the difference of response to gibberellin among tree species in relation to flower induction. In recent years, however, it is reported by many investigators that there is some interaction between gibberellin and auxin in the growth and differentiation of plants. Kuse[42] found in young sweet potato stems that gibberellin cannot produce its growth-promoting effect unless the tissue contains natural auxin or exogenous IAA. The author[7] also obtained the similar result on the growth of pine hypocotyl sections. Therefore, it is thought that the above-mentioned difference in endogenous auxin in conifers may have some connection with the difference in the response of tree species to exogenous gibberellin.

Studies on the relation between flower bud formation and endogenous growth substances are comparatively little. Applying the paper chromatography, Harada and Nitsch[43] studied changes in methanolic extractable growth substances during the flower initiating processes in the long-day plant *Rudbeckia speciosa*, the cold-requiring plant Japanese chrysanthemum variety Shuokan, and the short-day plant Shasta chrysanthemum. In the long-day and cold-requiring species, a new substance (substance E) appears in the extract of the apices when the plants become ready to bolt and flower. So they thought that the substance E is a cause rather than a consequence of the bolting phenomenon. Harada[44] also has shown that the level of a "substance E" increases markedly during flower induction and bolting in a long-day plant, *Nicotiana sylvestris*. Nitsch et al.,[45] however, reported that factors D and E in *Nicotiana*, which probably correspond to Harada's substance E, tended to decrease during actual flower initiation, as if they were used up by this process. Bouillenne-Walrand et al.,[46] on the other hand, have reported that the level of free IAA drops in the leaves of *Hyoscyamus niger* during the period of flower induction, to increase again temporarily at the time of bolting. Nitsch et al.[45] observed a similar variation of a substance resembling IAA in the shoot tip of *N. sylvestris*. Very recently, Kato[47] studied physiologically the flower head formation and development of cauliflower plants. He reported that auxins, especially in the Rf value of IAA, declined temporarily in the apical part of the plants immediately before flower head initiation, and that the content of gibberellin-like substances was small amount at the vegetative stage and increased considerably after flower head initiation. Skene and Lang[48] have studied the relation between native gibberellins and flower formation in the long-thort-day plant, *Bryophyllum daigremontianum*. Two zones of gibberellin-like activity were contained in the plants raised in long days, but not or slightly in those grown in short days. On plants shifted from long days to short days a rise in the level of an activity, which is likely to be gibberellin A_5, occurred during the pre-flowering period or after the appearance of flower primordia. Lang[49] has found a qualitatve and quantitative difference in acetone-extractable, gibberellin-like substances between induced

and noninduced annual *Hyoscyamus niger*. Purified extracts from the shoots of induced plants that had begun to bolt were found to contain appreciably more gibberellin-like substances than noninduced plants. The author[2] studied previously changes in endogenous growth substances occurring in relation to natural- or gibberellin-induced flower formation in *Cryptomeria japonica*, employing the paper chromatography and the *Avena* straight growth test. And he found that the flower induction of the plant seems to be closely associated with the reduction of auxin level, especially IAA, and with the increase of a promoting substance with Rf value greater than that of IAA in the new shoots.

In the present experiments assayed by the pine hypocotyl test and the rice seedling test, in every case of girdling and gibberellin treatments an auxin of the IAA position (Factor Ⅰ) in the acid fraction, auxins in the aqueous fraction, and gibberellin-like substances respectively decreased after the treatments, but inhibitor β increased on the contrary. Further a promoting substance of Rf 0.6～0.7 (Factor Ⅱ) in the acid fraction appeared suddenly at the time of floral initiation. The Rf value and the state of appearance of the Factor Ⅱ resemble closely those of Harada's "substance E", which has been detected *Rudobeckia speciosa*, *Chrysanthemum morifolium*, and *Nicotiana sylvestris*. As the Factor Ⅱ in *Cryptomeria japonica* appears only at the time of floral initation, it is doubtful whether the substance is a cause of floral initiation. However, the Factor Ⅱ is found in large quantities at the time of flower bud formation in the buds of *P. densiflora* which has attained reproductive maturity. It is imagined, therefore, that the substance may play some role in flower formation in conifers. The relationship between the fall of level of the Factor Ⅰ, water-soluble auxins and gibberellin-like substances by girdling or gibberellin treatment, and flower bud formation in *Cryptomeria* is not clear. The change of these substances, however, occurs prior to floral initiation, and also in *P. densiflora*, the content of water-soluble auxins is less in mature trees than in young seedlings. It is considered, therefore, that these substances also may have relations with flower formation in conifers. It is well known in short-day plants that photoperiodic induction of flowering is closely associated with reduced levels of auxin in the plant body. At the present time, plant hormones have been considered to play indirect roles in the floral initiaton process and probably to participate in flower formation through the synthesis of nucleic acids and enzymes.

Ⅳ. Relation between flower sex differentiation and endogenous growth substances in conifers

In *P. densiflora* and *P. thunbergii*, female strobiles generally grow at the apex of vigorous shoots, while male strobiles on the lateral side of the lower part of weak shoots. Such a difference of flower-bearing position due to flower sex is observed also in *Cr. japonica*. These habits are hereditary. In some *Pinaceae* species, however, female strobiles are sometimes found singly or in abundance on the lower lateral portion of new shoots, and male strobiles seldom at the tip of the new shoot. Fujii[50] observed that the development

of lateral female strobiles on *P. densiflora* occurred mostly on elongated shoots which had been changed from dwarf shoots by the injury of primary long shoots. From these facts, it is considered that the sexuality of strobiles is determined in different physiological conditions but it is controlled by a condition of great delicacy. This experiment was undertaken to make clear the relationship between flower sex differentiation and endogenous growth substances.

1. Materials and methods

In order to make clear physiologically that the sex expression of strobiles differs with the position of bearing of flower buds, in the spring and autumn of the year 1965 an experiment was performed using *P. thunbergii* and *P. densiflora*. For the extraction of auxin, buds bearing male strobiles and those bearing female ones were separately collected from trees of 5 to 20 years old in April, May and October. The lower part of the new shoot bearing male strobiles and the upper part bearing female ones were also collected from 5-year-old *P. densiflora* on April 25 (at the time of meiosis of PMCs). The following experiment, moreover, was undertaken to invesigate the relation between the sex transition of strobiles to female by pinching and endogenous growth substances. 5-year-old *P. densiflora* bearing male strobiles were chosen as the material of pinching. All of the new shoots on main trunk and vigorous branches were pinched off at the part just above the position bearing male strobiles (1/2~2/3 of the whole length) on March 29, 1965. After 28 and 49 days, the residual pinched shoots were gathered for extraction of auxin. The lower part of unpinched shoots were used as the control.

In the above experiments, 20 g of samples (in fresh weight) were used for extraction of auxin. Methods for the extraction, separation and bioassay of auxin were much the same as those in the first experiment (see Ⅱ-1). In general, the strip of developed chromatogram equivalent to the extract of 5 g samples was used for auxin bioassay,

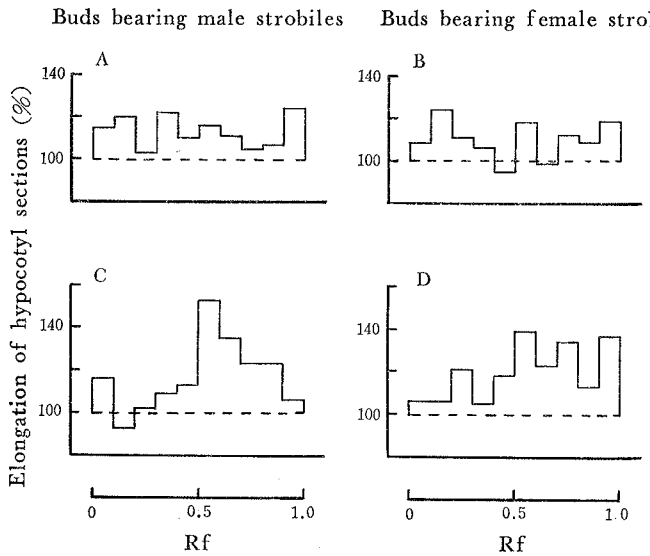

Fig. 17. Comparison of neutral auxins as contained in the male strobile bearing buds and female bearing ones of *P. densiflora*.

Left column, buds bearing male strobiles; right column, buds bearing female strobiles. A~B, *P. densiflora* (5 years old, April 18); C~D, *P. densiflora* (20 years old, May 14).

though on the chromatograms of the aqueous fraction analysed in April and May the strip corresponding to 2.5 g of samples was assayed.

2. Resuts

1) Relation between the position of bearing of male and female strobiles and endogenous growth substances in pines

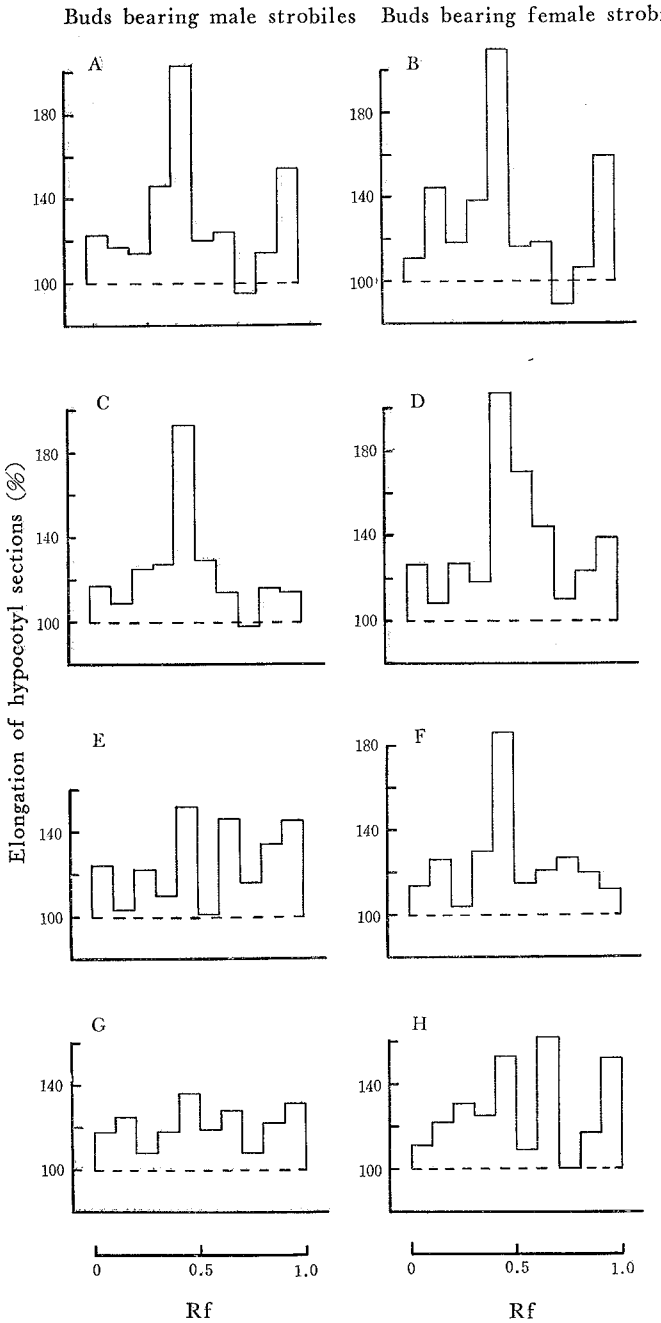

Fig. 18. Comparison of acid auxins as contained in the male strobile bearing buds and female bearing ones of *P. thunbergii* and *P. densiflora*.
Left column, buds bearing male strobiles; right column, buds bearing female strobiles. A~B, *P. thunbergii* (20 years old, October 8); C~D, *P. densiflora* (13 years old, October 16); E~F, *P. densiflora* (5 years old, April 18); G~H, *P. densiflora* (20 years old, May 14).

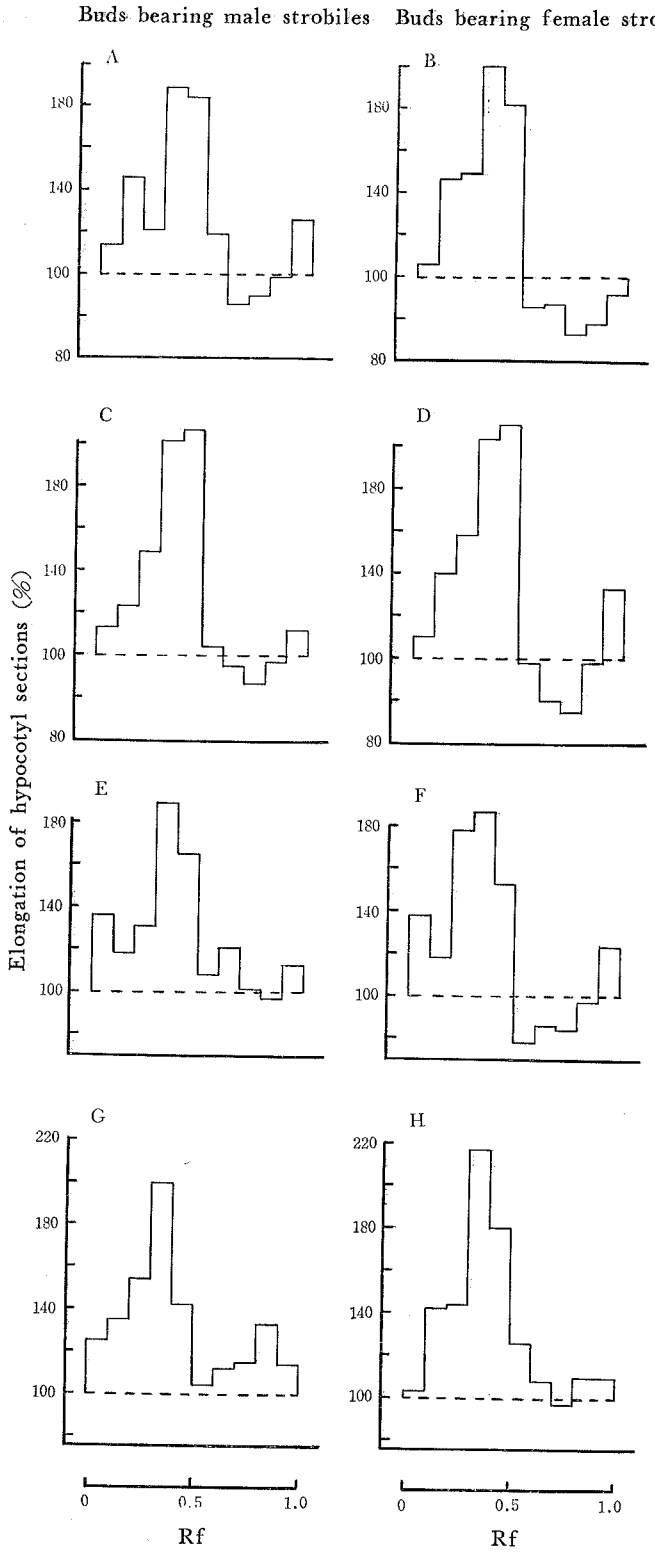

Fig. 19. Comparison of aqueous auxins as contained in the male strobile bearing buds and female bearing ones of *P. thunbergii* and *P. densiflora*. Other descriptions are the same as those in Fig. 18.

Table 6. Amounts of auxin in the male strobile bearing buds and female bearing ones of pines.

Species	Tree age	Date collected	Approximate concentration (μg. IAA equivalents/100 g.f.w.)								Remarks
			Neutral auxin		Acid auxin		Aqueous auxin		Total auxin		
			Male	Female	Male	Female	Male	Female	Male	Female	
Pinus thunbergii	20	Oct. 8	—	—	92.2	116.5	61.4	95.7	** (153.6)	** (212.7)	Formation of flower buds was externally recognized
P. densiflora	13	Oct. 16	—	—	52.6	114.2	168.5	256.5	** (221.1)	** (370.7)	
P. densiflora	5	Apr. 18	2.8	2.2	13.1	31.6	104.4	112.6	120.3	146.4	Time of formation of PMCs
* *P. densiflora*	20	May. 14	9.4	6.7	6.5	25.2	158.5	291.7	174.4	323.6	Time of meiosis of PMCs

* The samples were collected from a tree growing in Tottori University Forest at Hiruzen where the elevation is about 600 m.

** Showed a total of acid and aqueous auxins.

The comparison between auxins and inhibitors in male strobile bearing buds and those in female strobile bearing buds is shown in Figs. 17~19 and Table 6. In the neutral fraction, conspicuous promoting zones were not seen and consequently the amount of auxin was very little as compared with two other fractions. It seems therefore that there is no remarkable difference between the male strobile bearing buds and the female buds in the quality and quantity of neutral auxin.

Several promoting zones were present in the acid fraction. Among these, a zone of Rf 0.4~0.5 (Factor I) was most in evidence and was seen on every chromatogram, while on some chromatograms two other promoting zones with comparatively higher auxin activity were detectd at Rf 0.6~0.7 (Factor II) and Rf 0.8~1.0. Factor I was found in a relatively higher quantity in buds bearing female strobiles than in those bearing male strobiles. A similar tendency was also recognized in Factor II and an active zone of Rf 0.8~1.0, except for a case of *P. densiflora* collected on April 18. On small promoting zones having smaller Rf values than Factor I a definite result was not obtained, because the position of occurrence of the zones on chromatogram was variable. As shown in Table 6, however, the total amount of acid auxin was distinctly more in buds bearing female strobiles than in those bearing male strobiles.

In the aqueous fraction, a large promoting zone appeared in the region between Rf 0 and 0.6, and a broad inhibiting zone in the region between Rf 0.5 and 0.9. As listed in Table 6, the content of the aqueous auxin was greater in buds bearing female strobiles than in those bearing male strobiles. The similar difference in content between male and female was also observed in inhibitor β occurring at Rf 0.5~0.9.

The distribution of auxins and inhibitors in new shoots of *P. densiflora* at the time of meiosis of PMCs is shown in Fig. 20. In the acid fraction, a promoting zone was detected at Rf 0.4~0.6, while an inhibting zone was at Rf 0.7~0.8. On the other

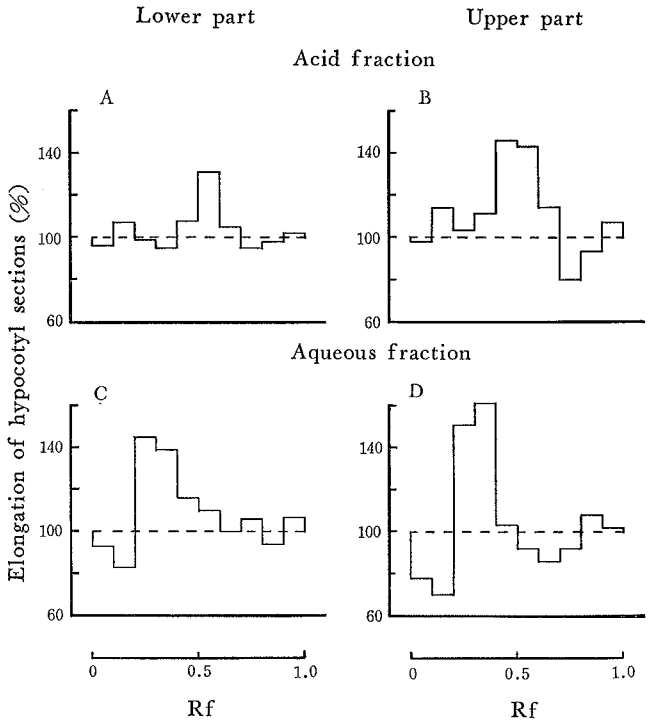

Fig. 20. Distribution of auxins and inhibitors in new shoots of *P. densiflora*.

A~B, acid fraction; C~D, aqueous fraction. A~C, lower part; B~D, upper part. The samples were collected from 5-year-old trees on April 25 (at the time of meiosis of PMCs).

hand, in the aqueous fraction a promoting zone was found at Rf 0.2~0.4, and two inhibiting zones at Rfs 0~0.2 and 0.5~0.8. Every one of these promoting and inhibiting zons had higher activity in the extract obtained from the upper part of new shoots than in that obtained from the lower part. Namely, the contents of auxin and inhibitor are greater at the upper part of new shoots bearing female strobiles than at the lower part bearing male ones.

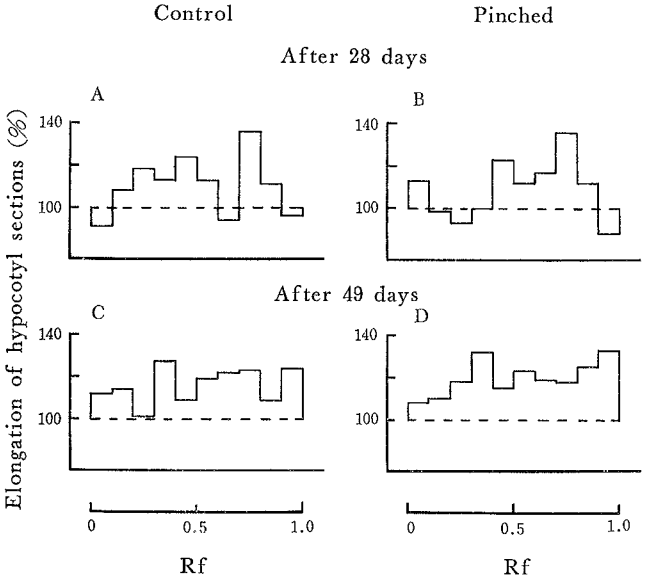

Fig. 21. Histograms showing changes in the auxin of neutral fraction contained in new shoots of *P. densiflora*, caused by pinching.

A and C, controls; B and D, pinched shoots. A and B, April 26 (28 days after pinching); C and D, May 17 (49 days after pinching).

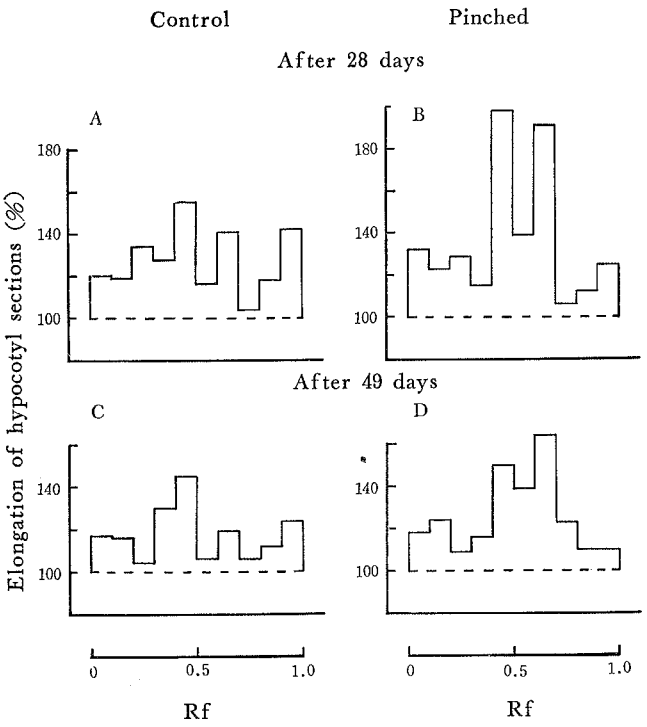

Fig. 22. Histograms showing changes in the auxin of acid fraction contained in new shoots of *P. densiflora*, caused by pinching. Other descriptions are the same as those in Fig. 21.

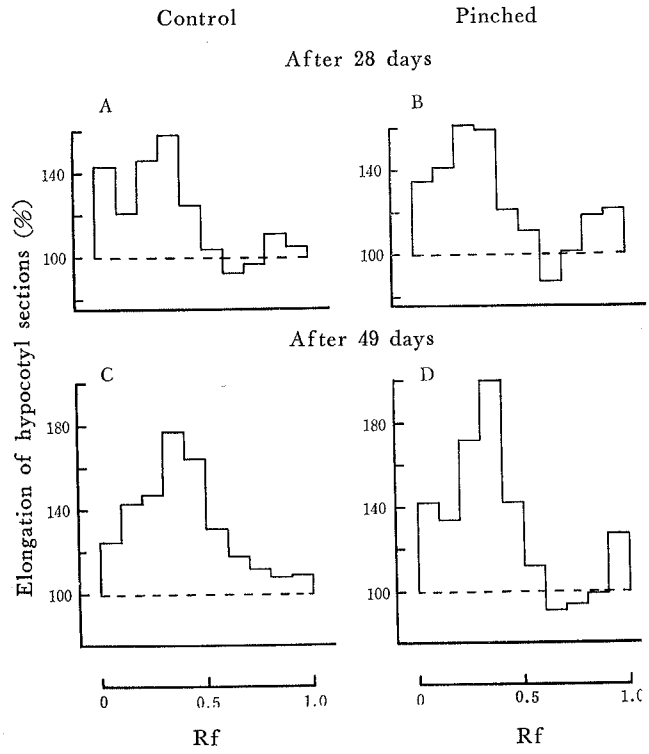

Fig. 23. Histograms showing changes in the auxin of aqueous fraction contained in new shoots of *P. densiflora*, caused by pinching. Other descriptions are the same as those in Fig. 21.

From these results, it may be considered that female strobiles are formed at the region of the tree having both auxin and inhibitor levels higher than male strobiles.

Table 7. Changes in amount of auxin in new shoots of *P. densiflora* caused by pinching.*

Date of collection	Approximate concentration (μg. IAA equivalents/100 g.f.w)								Remarks
	Neutral auxin		Acid auxin		Aqueous auxin		Total auxin		
	Control	Pinched	Control	Pinched	Control	Pinched	Control	Pinched	
After 28 days (April 26)	3.3	3.2	14.5	112.1	29.0	47.9	46.8	163.2	Time of meiosis of PMCs
After 49 days (May 17)	4.2	5.9	5.9	20.0	71.7	182.1	81.8	208.0	Flowering time

* The new shoots were pinched on March 29.

2) Changes of auxin in new shoots of *P. densiflora* caused by pinching

The experimental results are shown in Figs. 21～23 and Table 7. Auxins of the neutral fraction were hardly affected by pinching. However, auxins of the acid fracton, especially Factor Ⅰ (an active zone of Rf 0.4～0.5) and Factor Ⅱ (an active zone of Rf 0.6～0.7) increased rapidly after pinching. These increases were very remarkable on the 28th day after pinching. The Factor Ⅱ, however, showed far higher activity than the control even after 49 days from pinching. In the aqueous fraction, two promoting zones (Rfs 0～0.6 and 0.8～1.0) and an inhibiting zone (Rf 0.6～0.8) were detected. The activities of these promoting and inhibiting zones tended to be increased by pinching. Such a tendency was strongly in evidence in a promoting zone of Rf 0.2～0.4. It was seen from Table 7 that acid auxins increased rapidly, but aqueous auxins gradually after pinching.

From the above results, it may be said that the pinching treatment raises auxin levels in new shoots and brings about the physiological condition of new shoots to the state suitable for the expression of female strobiles.

3. Discussion

It is now well established that sex expression in various monoecious *Cucurbitaceae* may be modified by auxin treatment. Laibach and Kribben[51～52] have shown that the proportion of female flowers produced by *Cucumis sativus* may be substantially increased by treatment during early growth with IAA and NAA. Similar results have been obtained by Nitsch et al.[53] with squash, and by Wittwer and Hillyer[54] with cucumber. Arguing from their findings with cucumber, Laibach and Kribben[55] have suggested that the sexuality of flowers is dependent upon the concentration of native auxin available in the leaf axil during the period of flower formation, and if it is dense, more female flowers will come out. However, Ito and Saito[56～58] observed that in Japanese cucumber that not only auxins but also anti-auxins inhibit the male flower formation and stimulate the female flower differentiation when applied at higher concentrations depressing the stem

growth, while applied gibberellin invigorates the plant growth, promotes male flower formation and restricts the female flower differentation. Further, studying the relationship among the content of auxin and gibberellin in the stem apex of the plant, the plant growth, and the flower sex differentiation, they recognized that the female flower formation is accompanied with weak growth and lower auxin and gibberellin levels in the growing point. However, it is difficult to explain definitely the difference in the sex pattern display among the typical cucumber varieties by the mere content of auxin and gibberellin in the stem apex alone. Therefore, they concluded that flower sex in the cucumber plant is determined by the amount of the specific substances formed in the foliage leaves, and auxin and gibberellin in the stem apex will act on controlling the flow of the stream of the specific substances.

As regards conifers, Saito and Hashizume[59] were successful in causing artificial sex transition of lateral strobiles of *Pinus densiflora* and *P. thunbergii* to female by pinching the shoot above the portion bearing male strobiles or spraying with NAA over the shoot. Hashizume[4~5] also observed in *Cryptomeria japonica* that the sex transition of male strobiles to female can be induced by the dual treatment of pinching new shoots and spraying with gibberellin or by combining treatments of pinching and spraying with gibberellin and auxin. Recently, the author[40] found that female flower differentiation in *Larix leptolepis* is promoted by injecting NAA into the trunk immediately after flower bud formation. These results may suggest that auxin is closely connected with flower sex differentiation in conifers. In this experiment on *Pinus*, female strobiles are formed at the region of the tree having auxin levels higher than male strobiles. Also auxin levels in new shoots are raised by pinching which is effective in the sex transition of male strobiles to female. From these results, it may be concluded that in conifers female strobiles are formed in a condition of auxin levels higher than male strobiles. Mirov[60] studied the distribution of growth hormones in the developing shoots of ponderosa pine and Torrey pine by Went's *Avena* method. According to the results of his experiment, the lowest concentration of auxin was found at the uppermost 5 mm of the shoot. With the increase of the distance from the tip, the auxin concentration also increased and reached a maximum near the base of the present year's shoot. Onaka[61] came to the same conclusion in Japanese black pine shoot. These results do not coincide with those of the present experiment. This is probably due to the difference of experimental methods.

Summary

The present experiments were undertaken to ascertain growth substances existing in conifers for the purpose of explaining physiologically the difference in the response of tree species to flower induction by gibberellin, and to make clear the relation between flower bud formation or flower sex differentiation and endogenous growth substances in conifers. Auxins and gibberellin-like substances in shoots of conifers were extracted with methanol and separated by paper chromatography. Auxins were bioassayed by the pine

hypocotyl test and gibberellin-like substances by the rice seedling test.

1. Auxins and gibberellin-like substances present in the shoots of conifers

Both auxins and gibberellin-like substances were found in almost all species examined. When developed with ammoniacal *iso*-propanol, four kinds of auxin activities were detected in each of the neutral, acid and aqueous fractions. Among these, three promoting zones, found at Rfs 0.4~0.5 (Factor Ⅰ) and 0.6~0.7 (Factor Ⅱ) in the acid fraction and at Rf 0.2~0.5 (Factor Ⅲ) in the aqueous fraction, were especially remarkable. The Factor Ⅰ corresponded to the Rf value of IAA developed at the same time, but the others could not be identified. On the other hand, by the rice seedling test four gibberellin-like activities were found at Rfs 0~0.3, 0.4~0.5, 0.6~0.8 and 0.8~1.0, while a growth inhibiting activity was at Rf 0.5~0.8. The actvity of Rf 0.6~0.8 seems to be attributed to the known gibberellin A.

The shoots of conifers contained relatively higher concentrations of auxins and their total contents were equivalent to 41~286 μg IAA per 100 g fresh weight. The content of auxin in each fraction was abundant in the following order: aqueous fraction > acid fraction > neutral fraction. The amount of gibberellin-like substances in shoots of conifers was estimated to be equivalent to 0.03~1.63 μg gibberellin A_3 per 100 g fresh weight.

We compared endogenous growth substances between the flower-induced and non-flower-induced trees by gibberellin application. The two auxins located at Rf 0.5~0.8 in the neutral fraction and at Rf 0.6~0.7 in the acid fraction gave higher activity in the latter, especially in the *Pinus* species. On the water-soluble auxins, gibberellin-like substances and inhibitors, however, there were found no qualitative and quantitative differences between the two tree groups.

2. Relation between flower bud formation and endogenous growth substances

Considerable changes occurred on growth substances in new shoots of *Cr. japonica* in relation to flower induction by girdling or gibberellin treatment. Factor Ⅰ (Rf 0.4~0.5) in the acid fraction, water-soluble auxins and gibberellin-like substances tended to decrease after girdling or gibberellin treatment. On the contrary, Factor Ⅱ (Rf 0.6~0.7) in the acid fraction increased suddenly and rapidly at the time of flower initiation. On the other hand, growth inhibiting substances, especially inhibitor β, tended to increase after the treatments. Exogenous gibberellin A absorbed into the shoot of *Cr. japonica* usually was consumed in that condition in the shoot, but a part of it seemed to be converted into a glucoside when applied in large quantities.

It was shown that auxins in the bud of *P. densiflora* vary according to tree ages. The variation was remarkable on auxins of acid and aqueous fractions. Factors Ⅰ and Ⅱ, especially Factor Ⅱ, in the acid fraction were small in young seedlings and abundant in mature trees. Therefore, the content of acid auxins tended to increase with the increase of tree age. On the contrary, aqueous auxins had the highest concentration in young

seedlings.

From these results, it is suggested that endogenous growth substances in conifers may play some role in flower bud formation.

3. Relation between flower sex differentiation and endogenous growth substances

The content of auxin and inhibitor in pines was greater in the bud bearing female strobiles than in that bearing male strobiles. Likewise in the new shoots it was also greater in the female strobile bearing parts than in the male parts. The difference was distinctly recognized on the growth substances of acid and aqueous fractions.

The content of auxin in pine shoots also was increased by pinching new shoots, by which the sex transition of male strobiles to female is induced. The increase was especcially marked on Factors Ⅰ and Ⅱ in the acid fraction. Acid auxins increased rapidly, and aqueous auxins gradually after pinching.

From these results, it may be concluded that in conifers female strobiles are formed in a condition of auxin levels higher than male ones.

References

1) Hashizume, H. : The effect of gibberellin upon flower formation in *Cryptomera japonica*. *J. Jap. For. Soc.*, **41**, 375～381 (1959)

2) Hashizume, H. : 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 (1961)

3) Saito, Y. : Artificial control of sex differentiation in Japanese red pine and black pine strobiles. *J. Fac. Agr. Tottori Univ.*, **3**, 1～29 (1957)

4) Hashizume, H. : The effect of gibberellin upon sex differentiation in *Cryptomeria japonica* strobiles. *J. Jap. For. Soc.*, **42**, 176～180 (1960)

5) Hashizume, H. : The effect of gibberellin on sex differentiation in *Cryptomeria japonica* strobiles. Ⅱ. Effects of auxin and urea on gibberellin-induced sex transition to female in strobiles. *J. Jap. For. Soc.*, **43**, 47～49 (1961)

6) Srivastava, B. I. S. : Ether-soluble and ether-insoluble auxins from immature corn kernels. *Plant Physiol.*, **38**, 473～478 (1963)

7) Hashizume, H. : A method for auxin measurement using pine hypocotyl sections. *J. Jap. For. Soc.*, **47**, 304～312 (1965)

8) Murakami, Y. : The effect of the extract of immature bean seeds on the growth of coleoptile and leaf of rice plant. *Bot. Mag. Tokyo* **70**, 376～382 (1957)

9) Ogawa, Y. : Studies on the conditions for gibberellin assay using rice seedling. *Plant and Cell Physiol.*, **4**, 227～237 (1963)

10) Hashizume, H. : Gibberellin-like substances in new shoots of *Larix leptolepis* (Preliminary report). *Trans. Tottori Soc. Agr. Sci.*, **18**, 53～60 (1965)

11) Fransson, P. : Studies on a shoot and root cell elongation stimulator in *Pinus silvestris*. *Physiol. Plant.*, **12**, 188～198 (1959)

12) Allen, R. M. : Changes in acid growth substances in terminal buds of longleaf pine saplings

during the breaking of winter dormancy. *Physiol. Plant.*, **13**, 555~558 (1960)

13) Ueda, K. & Saito, T. : Separation of auxin and growth-inhibitors of coniferous trees and bamboo by paper partition chromatography. *Trans. 3rd Meet. Kansai Branch, Jap. For. Soc*, 13~14 (1953)

14) Ogasawara, R. : Studies on auxins and inhibitors in *Pinus thunbergii*. (II). Existence of indoleacetic acid. *Trans. Tottori Soc. Agr. Sci.*, **14**, 102~106 (1962)

15) Ogasawara, R. : Studies on auxins and inhibitors in *Pinus taeda* and *Pinus pinaster*. *Trans. Tottori Soc. Agr. Sci.*, **15**, 35~45 (1963)

16) Ogasawara, R. : Studies on auxins and inhibitors in *Pinus thunbergii*. *J. Jap. For. Soc.*, **43**, 50~54 (1961)

17) Ogasawara, R. : Studies on auxins and inhibitors in the buds of *Pinus strobus*. *J. Jap. For. Soc.*, **43**, 307~310 (1961)

18) Hashizume, H. : Changes in endogenous growth substances during flower initiation and sex differentiation in *Cryptomeria japonica*. *Trans. Tottori Soc. Agr. Sci.*, **12**, 150~154 (1960)

19) Yim, K. B. : Physiological studies on rooting of pitch pine (*Pinus rigida* Mill.) cuttings. *Rep. Inst. For. Gen. Korea*, **2**, 22~56 (1962)

20) Saito, Y. et al. : Changes in growth substances in the buds of *Abies sachalinensis* during sprouting season. *Trans. 13th Meet. Hokkaido Branch, Jap. For. Soc.*, 79~84 (1964)

21) Clark, J. & Bonga, J. : Evidence for indole-3-acetic acid in balsam fir, *Abies balsamea* (L.) Mill. *Canad. J. Bot.*, **41**, 165~173 (1963)

22) Hashizume, H. : Detection of auxins and inhibitors in the pine seeds and needles by the pine hypocotyl test. *J. Jap. For. Soc.*, **47**, 421~425 (1965)

23) Audus, L. J. & Gunning, B. E. S. : Growth substances in the roots of *Pisum sativum*. *Physiol. Plant.*, **11**, 685~697 (1958)

24) Gunning, B. E. S. : Auxin activity in ether-insoluble fractions of extracts of rhubarb petioles. *Nature*, **192**, 661~662 (1961)

25) Kato, J. et al. : Gibberellin-like substances in plants. *Nature*, **196**, 687~688 (1962)

26) Kurgman, S. L. : A gibberellin-like substance in immature pine seed. *For. Sci*, **13**, 29~37 (1967)

27) Ogawa, Y. : Gibberellin-like substances occurring in the seed of *Pharbitis nil* Chois. and their change in contents during the seed development. *Plant and Cell Physiol.*, **4**, 217~225 (1963)

28) Murakami, Y. : A paper chromatographic survey of gibberellins and auxins in immature seeds of leguminous plants. *Bot. Mag. Tokyo*, **72**, 36~43 (1959)

29) Ogawa, Y. : Changes in the content of gibberellin-like substances in the seed of *Prunus persica*. *Bot. Mag. Tokyo*, **78**, 412~416 (1965)

30) Murakami, Y. : Formation of gibberellin A_3 glucoside in plant tissues. *Bot. Mag. Tokyo*, **74**, 424~425 (1961)

31) Murakami, Y. : Occurrence of 'water-soluble' gibberellin in higher plants. *Bot. Mag. Tokyo*, **75**, 451~452 (1962)

32) Ogawa, Y. : Ethyl acetate-soluble and "water-soluble" gibberellin-like substatances in the seeds of *Pharbitis nil*, *Lupinus luteus* and *Prunus persica*. *Bot. Mag. Tokyo*, **79**, 69~76 (1965)

33) Tamura, S. et al. : Isolation of helminthosporol as a natural plant growth regulator and its chemical structure. *Agr. Biol. Chem*, **27**, 738~739 (1963)

34) Leopold, A. C. : Auxins and plant growth, Univ. of California Press, 63 (1955)

35) Phinney, B. O. & West, C. A. : Gibberellins as native plant growth regulators. *Ann. Rev. Plant Physiol.*, **11**, 411～436 (1960)

36) Murakami, Y. : The occurrence of gibberellins in mature dry seeds. *Bot. Mag. Tokyo*, **72**, 438～442 (1959)

37) Hirono, Y. et al. : Eine neue Methode für Gibberellin-test bei einem Zwergmutanten von *Pharbitis nil* Chois. *Plant and Cell Physiol.*, **1**, 81～89 (1960)

38) Kato, Y. et al. : Stimulation of differentiation of flower bud in conifer by gibberellin (I). *J. Jap. For. Soc.*, **41**, 309～311 (1959)

39) Shidei, T. et al. : Flower buds formation on Sugi (*Cryptomeria japonica*) and Metasequoia (*Metasequoia glyptostroboides*) by gibberellic acid treatment. *J. Jap. For. Soc.*, **41**, 312～315 (1959)

40) Hashizume, H. : Experimental induction of female flowers in young Japanese larch (*Larix leptolepis* Gordon). *J. Jap. For. Soc.*, **49**, 405～408 (1967)

41) Bennet-Clark, T. A. & Kefford, N. P. : Chromatography of the growth substances in plant extracts. *Nature*, **171**, 645～647 (1953)

42) Kuse, G. : Necessity of auxin for the growth effect of gibberellin. *Bot. Mag. Tokyo*, **71**, 151～158 (1958)

43) Harada, H. & Nitsch, J. P. : Changes in endogenous growth substances during flower development. *Plant Phsiol.* **34**, 409～415 (1959)

44) Harada, H. : *Rev. Gén. Bot.*, **69**, 201～301 (1962) (Cited from 45)

45) Nitsch, J. P. & Nitsch, C. : The induction of flowering in *Nicotiana*. Ⅲ. Variations in the level of endogenous growth substances. *Amer. J. Bot.*, **52**, 591～598 (1965)

46) Bouillenne-Walrand, M.et al. : *Bull. Soc. Roy. Sci. Liége*, **29**, 228～238 (1960) (Cited from 45)

47) Kato, T. : On the flower head formation and development of cauliflower plants. Ⅱ. Physiological studies on the flower head formation. *J. Jap. Soc. Hort. Sci.*, **34**, 49～56 (1965)

48) Skene, K. G. M. & Lang, A. : Native gibberellins and flower formation in *Bryophyllum daigremontianum*. *Plant Physiol.*, **39** (Suppl.), 37 (1964)

49) Lang, A. : *Proc. Intern. Congr. Biochem.*, 4th Congr., Vienna, 1958, 126～140 (1959) (Cited from 35)

50) Fujii, K. : Physiological researches on the sexuality of the flowers of *Pinus densiflora* Sieb. et Zucc. *Bot. Mag. Tokyo*, **9**, 271～275 (1895)

51) Laibach, F. & Kribben, F. J. : Der Einfluß von Wuchsstoff auf die Bildung männlicher und weiblicher Blüen bei einer monözischen Pflanze. *Ber. Deutsch. Bot. Ges.*, **62**, 53～55 (1950)

52) Laibach, F. & Kribben, F. J. : Der Einfluss von Wuchsstoff auf die Blütenbildung der Gurke. *Naturwiss.*, **37**, 114～115 (1951)

53) Nitsch, J. P. et al. : The development of sex expression in cucurbit flowers. *Amer. J. Bot.*, **39**, 32～43 (1952)

54) Wittwer, S. H. & Hillyer, I. F. : Chemical induction of male sterility. *Science*, **120**, 893 (1954)

55) Laibach, F. & Kribben, F. J. : Die Bedeutung des Wuchsstoffs für die Bildung und Geschlechtsbestimmung der Blüten. *Beitr. Biol. Pflanz.*, **28**, 131～144 (1951)

56) Ito, H. & Saito, T, : Facotors responsible for the sex expression of the cucumber plant. XII. Physiological factors associated with the sex expression of flowers. *Tohoku J. Agr. Res.*, **11**, 287～308 (1960)

57) Saito, T. & Ito, H. : Factors responsible for the sex expression of the cucumber plant.

XIII. Physiological factors associated with the sex expression of flowers. (2). Role of gibberellin. *J. Jap. Soc. Hort. Sci.*, **32**, 278～290 (1963)

58) Saito, T. & Ito, H. : Factors responsible for the sex expression of the cucumber plant. XIV. Auxin and gibberellin content in the stem apex and the sex expression of flowers. *Tohoku J. Agr. Res.*, **14**, 227～239 (1963)

59) Saito, Y. & Hashizume, H. : Physiological researches on the sex differentation of Japanese red pine strobiles and its application to forest practice. *In Symposium in Japanese red pine, Kansai Jap. For. Soc.*, 91～94 (1954)

60) Mirov, N. H. : Distribution of growth hormone in shoots of two species of pine. *J. For.*, **39**, 457～464 (1941)

61) Onaka, F. : The effects of defoliation, disbudding, girdling and other treatments upon growth, especially radial growth in evergreen conifers. *Bull. Kyoto Univ. For.*, **18**, 55～95 (1950)

和 文 要 約

針葉樹の葉条に存在するオーキシンおよびジベレリン様物質とそれらの花芽分化，花性分化に対する役割

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針葉樹の葉条に含まれるオーキシンおよびジベレリン様物質をペーパー・クロマトグラフィーと生物試験法を併用して調べた。さらに内在生長物質と花芽分化および花性分化の関係について検討した。

1. 針葉樹の葉条に存在するオーキシンおよびジベレリン様物質

供試9樹種のほとんどすべてで，オーキシンおよびジベレリン様物質の存在が確認された。アンモニア性イソプロパノールで展開した場合，中性分画，酸性分画，水溶性分画のそれぞれで4種類のオーキシンが検出された。これらのうち，酸性分画の Rf 0.4～0.5 (Factor Ⅰ) と Rf 0.6～0.7 (Factor Ⅱ)，および水溶性分画の Rf 0.2～0.5 (Factor Ⅲ) の三つの促進帯がとくに顕著であった。Factor Ⅰ は IAA と推定されたが，他の促進帯は同定できなかった。他方，イネ苗試験法により4種類のジベレリン様物質 (Rf 0～0.3，0.4～0.5，0.6～0.8，0.8～1.0) と1種類の抑制物質 (Rf 0.5～0.8) が検出された。Rf 0.6～0.8のジベレリン様物質は既知のジベレリン A と Rf 値が一致する。

針葉樹の葉条には比較的高濃度のオーキシンが含まれている。その含量は IAA に換算すると葉条100 g 当たり41～286 μg に相当した。オーキシンの含量は中性分画＜酸性分画＜水溶性分画の順に大であった。ジベレリン様物質は葉条100 g 当たり0.03～1.63 μg GA_3 当量含まれていた。

ジベレリンで開花が誘起される樹種と誘起されない樹種の生長物質を比較すると，中性分画の Rf 0.5～0.8 と酸性分画の Rf 0.6～0.7の二つのオーキシンは，後者とくにマツ属の樹種に多いようであった。水溶性オーキシン，ジベレリン様物質および抑制物質は両者の間に著しいちがいが認められなかった。

2. 花芽分化と内在生長物質との関係

環状剝皮あるいはジベレリン処理による花芽分化の誘起に関連して，スギの新条内の生長物質に注目すべき変化がみられた。酸性分画の Factor Ⅰ，水溶性オーキシンおよびジベレリン様物質は処理後減少の傾向にあった。これに反し，Factor Ⅱ は花芽分化期に急激に増加した。また抑制物質，とくに inhibitor β は処理

後増加の傾向にあった。葉面散布などによって吸収されたジベレリンは葉条の中で直接消費されるが，大量に施与された場合には，その一部がグルコシドに転化するようである。

アカマツの芽に含まれるオーキシンは樹齢によって変化することがわかった。酸性分画の Factor Ⅰ と Factor Ⅱ は若い苗木に少なく，成熟木に多く含まれていた。とくに Factor Ⅱ はこの傾向が顕著であった。したがって，酸性分画のオーキシンの含量は樹齢の増加にともなって増大する。これに反し，水溶性オーキシンは若い苗木に最も多く含まれていた。以上の結果から，内在生長物質は花芽の形成になんらかの役割を演じているものと思われる。

3. 花性分化と内在生長物質との関係

マツに存在するオーキシンおよび抑制物質は，雄花を着生した芽あるいは新条の下部よりも雌花を着生した芽あるいは新条の上部に多いようであった。このちがいは酸性分画と水溶性分画の生長物質で顕著に認められた。新条におけるオーキシンの含量は雄花の雌性化に有効な摘心処理によって増加した。摘心による増加は酸性分画の Factor Ⅰ と Factor Ⅱ，水溶性分画の Factor Ⅲ で顕著であった。酸性分画のオーキシンは摘心後急激に，水溶性分画のオーキシンは緩慢に増量した。これらの結果から，雌花は雄花よりもオーキシン・レベルの高い条件のもとで形成されるものと思われる。